JOURNAL OF AGRICULTURAL RESEARCH

VOL. 39 WASHINGTON, D. C., AUGUST 15, 1929 No. 4

INFLUENCE OF ATMOSPHERIC AND SOIL MOISTURE UPON SEED SETTING IN RED CLOVER[1]

By E. A. HOLLOWELL

Assistant Agronomist, Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Red clover (*Trifolium pratense*) is mainly self-sterile, and cross-pollination and fertilization are dependent upon insect visits. The present study was made to determine whether high atmospheric humidity prevents fertilization and whether a deficiency or excess of soil moisture adversely affects the setting of seed. The difficulty of controlling soil moisture, the self-sterility of red clover, the heterogeneity of its genetic constitution, the method of flowering, and the arrangement and number of florets on a head all added to the complexities of the problem involved in this study.

REVIEW OF LITERATURE

The fertilization of red-clover florets is dependent, among other things, on the healthy germination of the pollen, and it has been thought that the stigmatic surface supplies the moisture necessary for this process. A consideration of the germination of pollen under different conditions is of primary importance in this study. Lidforss (*3*, *4*)[2] has contributed to the knowledge of the germination of pollen of various species.

Hansgirg (*1*), Martin (*5*), and Westgate and Coe (*9*) concluded from their experiments that red-clover pollen bursts immediately when immersed in water. The results shown by Williams (*10*) differ from those of the above-named investigators in that the bursting was not immediate, some grains resisting the action of the water for more than an hour.

Westgate and Coe (*9*) concluded that pollination did not take place when the flowers were wet. Williams (*10*) secured fertilization, however, after immersing the pollen grains in water for five minutes and then applying them to the stigmatic surfaces before they had dried, but when they were allowed to dry for an hour no fertilization occurred.

Martin (*5*) showed that artificial pollen germination is delicately adjusted to the absorption of water. He found that high atmospheric humidity and a germinating medium containing an optimum degree of moisture were essential for germination and concluded that the function of the stigma is to supply water. Martin further suggested that the quantity of water available to the plant may

[1] Received for publication Jan. 28, 1929; issued August, 1929. Contribution from the Department of Agronomy, Illinois Agricultural Experiment Station, and the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. This paper was submitted to the faculty of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Reference is made by number (italic) to "Literature cited," p. 247.

increase the moisture content of the stigmatic surface, thereby preventing the pollen from functioning properly. The supply of atmospheric moisture would be important if a balance between the water absorbed and that lost by transpiration were necessary for germination.

Westgate and Coe (9) stated that infertile ovules occurred in red clover and that moist soil and atmosphere appeared to be directly correlated with the degree of infertility. These investigators also stated that in first growth many red-clover plants produced 100 per cent infertile ovules. Williams (*10*) concluded that rainfall and temperature were responsible for a decrease in seed setting in some of his self-pollination studies, but he did not state whether the effect was direct or indirect. Martin (*5*) also pointed out that the poor seed crop sometimes harvested from the first growth may be related to the abundance of moisture usually present when the first crop is in bloom. While Schlecht (*6*) failed to obtain a large number of seeds, he concluded that high atmospheric humidity did not prevent the functioning of the pollen.

Welton and Morris (*8*) studied the effect of rainfall on the vegetative growth of red clover and reported that a positive correlation exists between rainfall and vegetative growth.

GREENHOUSE AND LABORATORY STUDIES

POLLEN GERMINATION

Martin (*5*) succeeded in germinating red-clover pollen and found that the necessary requirements were a nearly saturated atmosphere and a medium or base containing a specific quantity of water. Various solutions proved to be unsuited for such experiments. His results lack uniformity, which he attributed to the variable physical properties of the base.

An attempt was made by the writer to perfect a technique for testing the germination of red-clover pollen. Pieces of sheet gelatine were soaked in various concentrations of sucrose solution and pollen was placed on them. The results, however, were not consistent. In some cases satisfactory germination was recorded; in others negative results were obtained.

EFFECT OF WATER AND SUCROSE SOLUTIONS ON POLLEN

The results obtained by immersing pollen grains in water and sucrose solutions agree with those of Williams (*10*) that many grains burst immediately after immersion. Bursting occurred in two ways, by a rapid emergence of the protoplasmic contents through small openings in the cell walls and by a uniform collapsing of the cell. Some of the pollen grains collapsed after they had been in the liquid for a few minutes while others were not affected at the end of 10 minutes. Bursting was retarded to a greater extent on gelatine which had been soaked in a 30 per cent solution of sucrose than in a solution of lower concentration.

EFFECT OF CONTROLLED ATMOSPHERIC AND SOIL MOISTURE ON THE SETTING OF RED-CLOVER SEED

In the greenhouse, flowering red-clover plants afford an opportunity to investigate the viability of the pollen and the receptivity of the stigmatic surface by cross-pollinations. Such a study was carried on under conditions where the atmospheric humidity was controlled and its effect on seed production observed.

In this work, red-clover plants from spring and fall seedings were dug, potted in the field, and transferred to the greenhouse in October, 1926. By the use of artificial illumination, many plants were brought into bloom by the middle of December. Williams (*10*) has shown that the optimum time for pollination in the field is when the florets are first opened, but under the conditions prevailing in the green-

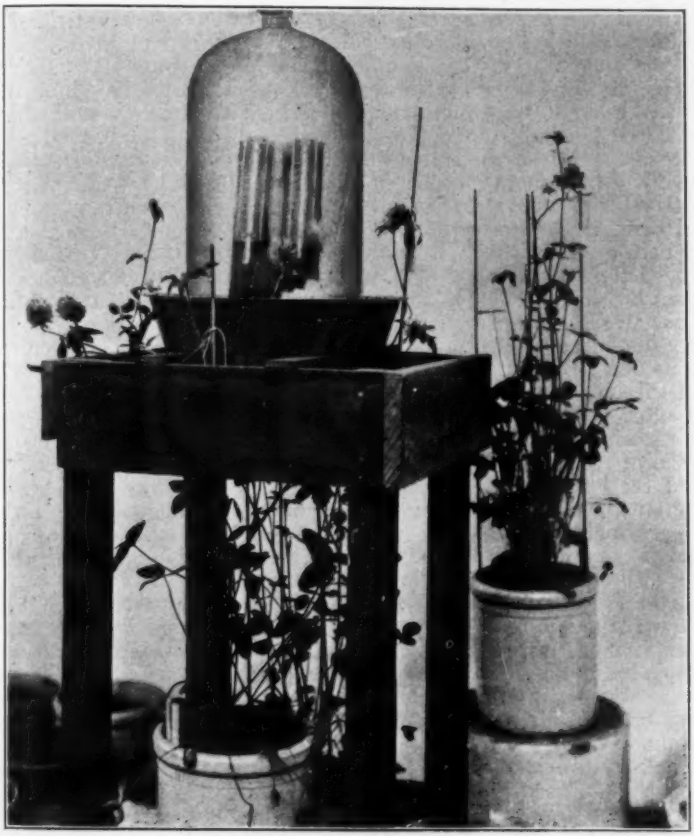

FIGURE 1.—Apparatus used in maintaining high atmospheric humidity for the determination of its effect on seed setting of red-clover plants. Urbana, Ill., 1926

house, the writer found it necessary to wait until the heads were more than one-third in bloom before the flowers were manipulated, as the anthers did not freely dehisce in the early blooming stage.

In order to study the effect of high atmospheric humidity, an apparatus was constructed, consisting of a wooden stand, a wet and dry bulb thermometer, a bell jar, and a tin pan having a depth of 3 and a diameter of 13 inches. (Fig. 1.) Four holes, each 1½ inches in diameter, were cut in the bottom of the pan, and over each hole was soldered a pipe 2 inches long which extended from the bottom of the pan upward. Water placed in this pan for humidification of the

atmosphere did not come in contact with any part of the plant. Flowering stems from different plants were carefully pushed through the pipes, cotton packed around them, and the whole covered with a bell jar. A saturated atmosphere was secured in the bell jar at the end of 10 minutes. The heads of the plants were usually projected into the bell jar 24 hours before pollination. When the effect of soil moisture was to be investigated, the quantity of water supplied to the plants was altered several days before pollination in order that a uniform condition might be established.

Experiments were also conducted under greenhouse conditions where the air was not saturated, but where the relative humidity usually did not vary more than 10 per cent. In cross-pollinating the florets, a small crooked needle roughened with a file proved to be more convenient and effective than camel's-hair brushes, small sticks, needles of the usual types, or the card method used by Williams (*10*). In tripping the flowers, the needle was inserted between the vexillum and carina. The lower part of the carina was gently pressed with the needle which was pulled upward at the same time. This action tripped the flower, and the pollen was thrown out against the needle which brushed the pistil, thus both gathering and depositing pollen in one stroke. To prevent as far as possible the deposition of pollen from the anthers to the stigma of the same floret, the pollen from the florets of a given plant was permitted to fall on one side of the needle, and the pollen from the other plant similarly on the opposite side. An effort was made to brush the stigmas of the florets in each case with foreign pollen. The time required for the manipulation of the florets of two heads varied from 10 minutes to one hour, depending upon the ease with which the work was done and the number of florets pollinated. The florets were usually manipulated three times at each pollination. After the last pollination, the manipulated heads were ordinarily left in the bell jar at least four days, or until all the corolla tubes had withered.

In these investigations the soil moisture was maintained about as follows: High moisture, where the soil was continually saturated; medium moisture, where a quantity of water was applied which seemed optimum for vigorous growth and the maintenance of a good physical condition of the soil; and low moisture, where the quantity of water available to the plant was barely sufficient to keep it from wilting.

The close proximity of the florets and the fact that it was necessary to carry out the pollinations in as short a time as possible made it difficult to determine accurately the number of florets manipulated on each head. In all, 18 heads were cross-pollinated, and as shown in Table 1, the reciprocal crosses yielded roughly the same number of seeds. When the heads were ripe, the seeds were removed and counted.

After the seed was harvested and the old growth removed, new growth rapidly developed. The plants began to produce flower heads by the latter part of March, 1927. Additional tests similar to those already described were conducted after a sufficient number of flower heads had developed. (Table 2.) In the second tests, the plants were not illuminated at night. To prevent the occurrence of high temperatures within the bell jar, it was necessary to place a shade over the apparatus when the sun was shining.

TABLE 1.—*Effect of atmospheric and soil moisture on the seed setting of red clover, artificially cross-pollinated under controlled greenhouse conditions at Urbana, Ill., December and January, 1926–27*

Plant letters and head numbers		Moisture				
Female parent	Male parent	Soil	Atmospheric, average relative humidity	Average temperature	Times pollinated	Seeds set
			Per cent	*°F.*	*Number*	*Number*
L-1	G-2	High	100	70	1	17
G-2	L-1	do	100	70	1	[a] 0
C-4	Selfed	do	100	70	2	0
C-5	C-6	do	100	70	1	0
C-6	C-5	do	100	70	1	0
G-1	H-1	do	55	65	1	22
H-1	G-1	do	55	65	1	38
A-1	B-1	Medium	100	70	1	52
B-1	A-1	do	100	70	1	[b] 0
C-1	D-1	do	53	65	1	25
D-1	C-1	do	53	65	1	16
E-1	C-2	do	53	65	1	5
C-2	E-1	do	53	65	1	12
F-1	E-2	do	53	65	1	32
E-2	F-1	do	53	65	1	20
F-2	I-1	do	53	65	1	25
I-1	F-2	do	53	65	1	32
E-3	I-2	do	53	65	1	15
I-2	E-3	do	53	65	1	[b] 0
B-2	Selfed	do	53	65	2	[b] 0

[a] Head became wilted and browned immediately after pollination, probably the result of rough manipulation.

[b] Plants accidently frosted before seed was formed.

TABLE 2.—*Effect of atmospheric and soil moisture on the seed setting of red clover, artificially cross-pollinated under controlled greenhouse conditions at Urbana, Ill., March, April, and May, 1927*

Plant letters and head numbers		Moisture				
Female parent	Male parent	Soil	Atmospheric, average relative humidity	Average temperature	Times pollinated	Seeds set
			Per cent	*°F.*	*Number*	*Number*
C-1	D-1	High	100	70	3	40
D-1	C-1	do	100	70	3	15
C-2	D-2	do	100	70	3	78
D-2	C-2	do	100	70	3	76
B-1	G-1	do	100	70	1	27
G-1	B-1	do	100	70	1	9
G-2	Selfed	do	100	70	1	0
B-5	do	do	100	70	1	0
C-3	D-3	do	55	70	2	42
D-3	C-3	do	55	70	2	23
E-1	C-5	do	55	70	1	9
C-5	E-1	do	55	70	1	13
C-4	Selfed	do	55	70	2	0
D-4	do	do	55	70	2	0
B-2	do	do	55	70	1	0
G-3	do	do	55	70	1	0
B-4	L-2	Medium	100	78	2	16
L-2	B-4	do	100	78	2	28
J-2	A-1	do	100	78	1	30
J-3	A-1	do	100	78	1	36
A-1	J-2	do	100	78	1	62
H-4	C-6	do	72	75	2	28
C-6	H-4	do	72	75	2	33
B-3	H-1	do	55	70	1	26
H-1	B-3	do	55	70	1	37
L-3	H-3	do	55	70	1	56
H-3	L-3	do	55	70	1	16
H-2	I-1	do	55	65	2	15
I-1	H-2	do	55	65	2	33
I-2	C-7	do	55	68	1	57
C-7	I-2	do	55	68	1	61
I-3	L-4	Low	80	70	1	21
L-4	I-3	do	80	70	1	21

In considering the number of seeds set, as given in Tables 1 and 2, it must be borne in mind that under field conditions an average of 25 seeds per head is considered to assure a fair seed crop.

In these experiments seeds in excess of this number were produced under conditions of high and of low atmospheric humidity and this even when the plants were growing in wet soil and the heads were in a saturated atmosphere.

The results of these investigations show, therefore, that high and low soil moisture and high atmospheric moisture do not prevent the setting of red-clover seed. Although considerable variation exists in the number of seeds set, the difference in the number produced under the various environments can not be interpreted as resulting from any one specific factor.

As has been pointed out, it was not possible to record the number of florets pollinated, and it is probable that foreign pollen was not transferred to every stigma. These facts render a strict statistical interpretation of the results impossible. It nevertheless remains true that seed was set under all conditions and in most cases as freely as could be expected in open-pollinated flowers in the field. There appears to be no material variation in the number of seeds set by reason of difference in atmospheric and soil moisture. Although most of the foregoing tests were conducted under extreme conditions of moisture, it is believed that intermediate conditions would give similar results.

If the florets had been in bloom for some time before they were pollinated, some ovules might have disintegrated before the pollen tubes reached them. This may have occurred in the case of heads C–1 and D–1, shown in Table 2. C–1, the female parent, produced nearly three times as many seeds as D–1. These heads were pollinated at three different times, head D–1 being approximately three-fourths mature when first pollinated, while C–1 was less than one-half in bloom. When recording the number of seeds set, it was noted that the seeds in C–1 were uniformly distributed throughout the head, whereas those of D–1 were located in the upper portion of the head, resulting in the setting of a decidedly smaller number of seeds. It seems probable that the ovules in the older flowers of D–1 had disintegrated before the pollen tubes reached them.

STUDIES UNDER CONTROLLED SOIL-MOISTURE CONDITIONS

SOIL MOISTURE AS A FACTOR IN GROWTH OF RED CLOVER

The quantity of seed produced by a given field of red clover will depend upon the number of flower heads, the number of florets, the number of florets pollinated, and the proportion of those pollinated that set seed. Soil moisture may affect any or all of these factors except the number pollinated. Under natural conditions this will depend, of course, upon the number and activity of the pollinating insects. The number of flower heads and the number of florets produced may be considered as an expression of the vigor of the plant as influenced by the moisture content of the soil. This may or may not have any relation to the effect of soil moisture on the effectiveness of pollen when applied to the stigma.

In the present study consideration was given to all of these characters, viz: The number of flowering stems and their height, as express-

ing vigor of growth; the number of flowering heads and the number of florets per head, as indicative of potentialities for seed production; and the number of florets that produced seed after being artificially pollinated, as showing the effect of various soil-moisture conditions on the receptivity of the stigmas.

During January, 1927, 27 galvanized-iron cans, 30 inches deep and 12 inches in diameter, painted with acid-proof enamel, were each filled with 135 pounds of the surface soil of Muscatine silt loam, uniformly compacted. Six additional cans were similarly filled with dune sand. Triplicate moisture samples of each soil type were taken as the cans were being filled, the loam containing 30.4 per cent and the sand 4.4 per cent moisture. The water-holding capacity of the

FIGURE 2.—Shelter used in maintaining soil moisture. Urbana, Ill., 1927

loam, as determined by the Hilgard method (*2*), was 85 per cent of the dry weight, and that for the sand was 33 per cent.

In order to avoid wide variations in seed production due to the genetic constitution of the plants, the seed used in these experiments was harvested in 1926, from two vigorous red-clover plants. The seeds were sown during the latter part of February, 1927, and the cultures were placed in the greenhouse where an approximate temperature of 60° F. was maintained. The same quantity of water, which was slightly below that needed for rapid growth, was applied to each can. On April 1, the plants were thinned to five per can, and later they were gradually hardened off. During the latter part of May they were placed under an especially constructed shelter, shown in Figure 2. In order more nearly to approximate natural conditions, the shelter was roofed with "Cel-o-Glass," which permitted the entrance of various wave lengths of the spectrum. In an effort to maintain more uniform soil temperatures, the cans were placed in sections of embedded draintiles.

On June 1, the moisture content of the soil in the cans was altered to low, medium, and high. While the capillary capacity as determined by the Hilgard method was not accurate, an approximation was secured as a guide by which the changes in soil moisture of each soil type could be made, as follows: High soil moisture, represented by the capillary capacity; medium soil moisture, 60 per cent of the water-holding capacity; and low soil moisture, 25 per cent of the water-holding capacity. After sufficient time had elapsed for moisture in the soil to approach a state of equilibrium, those cultures representing a high soil-moisture condition were supersaturated. The quantity of moisture applied to the soil of the other two sets appeared to be satisfactory. The quantity of moisture applied to the high-moisture series was therefore arbitrarily lowered until the water applied covered the surface of the soil approximately to a depth of one-fourth inch. A sufficient number of cans, filled with Muscatine silt loam were available for eight replications of each soil-moisture condition during the first growth of the plants. Duplicate tests of low, medium, and high soil moisture were conducted with cans filled with dune sand.

The cans were weighed two or three times a week, depending upon the prevailing environmental conditions, and the necessary water to correct for the quantity lost was added to the soil surface by spraying. The plants of the first growth were harvested individually August 3, 4, and 5, being cut 1½ inches above the surface of the soil. Records were taken of each flowering stem as to its height, the number of flower heads and their maturity, the size of leaf and head, the color of leaf, and the vigor of the plant. Moisture-free weights were determined for each plant.

Influence of Soil Moisture on Development of Red-Clover Plants

The foliage of the plants in soil of high moisture content became light green in color soon after they were subjected to the high moisture condition. This coloration of the leaves gradually increased; many became brown and others yellowish green by the time the plants were harvested. The leaves were also decidedly few and small when compared with leaves of plants grown under conditions of medium soil moisture. Adventitious roots developed from the lowest nodes of many flowering stems. The plants were markedly stunted, and the growth was lacking in vigor. The flower heads began blooming more than a week earlier than those on plants growing in soil of medium moisture.

Plants growing in soil of medium moisture developed vigorously, producing many flowering stems, medium-sized leaves normal in color, and large flower heads. The blooming of the flower heads was later than that of plants growing in soil kept at a high or a low soil-moisture content.

Plants growing in soil low in moisture were stunted, the leaves were of average size, but the heads appeared to be smaller than those from plants growing under a medium soil-moisture condition. Three weeks after the moisture content in this soil was lowered, the leaves showed symptoms of wilting during the day. This condition was more pronounced in the lower leaves, many of which turned brown. When the soil became dry enough to cause wilting, the green color of the plants became darker than normal.

Plants growing in dune sand maintained at soil moistures comparable to those used in the loam, developed similar external characteristics, as illustrated in Figure 3. The number of flower stems, number of heads, height of stems, and extent of vegetative growth were found to be adversely affected by extremely low or high soil moisture, as shown by Table 3.

Significant increases of the measured characters resulted from medium soil moisture when compared with low and high soil moisture.

FIGURE 3.—Effect of soil moisture on the development of red-clover plants grown in Muscatine silt loam (A) and in dune sand (B) at Urbana, Ill., 1927

No significant differences were observed in the effects of low and high soil moisture on the production of flower stems and the average height of the plants.

TABLE 3.—*Effect of low, medium, and high soil moisture on the production of flower stems and heads, and weight of dry material of red-clover plants grown in Muscatine silt loam and dune sand at Urbana, Ill., 1927*

Soil type and soil-moisture condition	Plants	First growth, average per plant of—			
		Flower stems	Height of stems	Heads	Weight of dry material
Muscatine silt loam:	*Number*	*Number*	*Inches*	*Number*	*Grams*
Low	45	4.7±0.20	10.1±0.37	11.5±0.83	5.45±0.227
Medium	45	7.5± .27	17.9± .41	36.5±2.08	13.66± .578
High	43	3.8± .17	11.9± .56	4.0± .38	3.21± .188
Dune sand:					
Low	9	5.7± .51	9.7± .54	11.3±1.80	4.99± .509
Medium	10	7.2± .25	17.0± .44	29.2±3.21	12.41± .828
High	10	3.2± .37	11.7±1.10	3.7± .54	[a]2.69± .371

[a] Based on 9 plants.

Even though the effect of low and high soil moisture was much the same on all these characters, yet in general a low soil-moisture content was less injurious than a high one. The effect of soil moisture was the same whether the plants were growing in Muscatine silt loam or dune sand, except that in dune sand there was no significant difference in the number of flower stems in plants growing in soil of low moisture as compared to soil of medium moisture, but plants growing in dune sand kept at a high moisture content produced fewer flowering stems. This lack of difference between plants in soil of low and soil of medium moisture content probably resulted from the fact that one plant was missing from one of the low-moisture cans, giving the other four more available water for development. Dune sand under the existing condi-

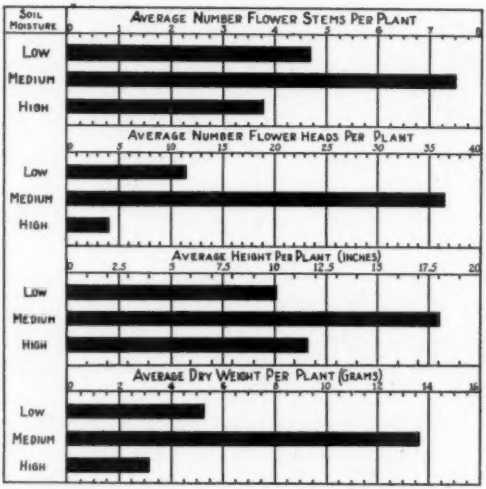

FIGURE 4.—Effect of soil moisture on the growth of red-clover plants in Muscatine silt loam, Urbana, Ill., 1927. (Based on Table 3)

tions was nearly as productive as the Muscatine silt loam, as is shown in Figures 4 and 5. The maximum productivity of either soil, however, was probably not reached. It is apparent that plants growing under optimum soil-moisture conditions will produce a greater yield of seed than those growing under more adverse conditions, provided the other factors that influence the setting of the seed are similar.

Influence of Soil Moisture on Numbers of Florets per Head

Plants growing in loam or in sand, low or high in moisture, appeared to develop smaller heads than plants growing in soil of medium moisture content. In order to test the correctness of this observation, the number of florets per head was determined for five mature heads from each of two different mother plants growing in the same can. A statistically significant difference in the number of florets per head was noted in favor of heads produced by plants growing in loam soil, low or medium in moisture content in comparison with those produced by plants growing in saturated soil. (Table 4.) Although the figures show a difference in the number of florets between plants

growing in soil of low moisture as compared to those in soil of medium moisture, calculated odds indicate that the difference is not significant. In dune sand a significant difference in number of florets per head occurred only between plants in soil of medium and those in soil of high moisture. Since the heads appeared smaller on plants in soil low in moisture, this was probably due to a lack of turgor in the cells of the corolla tissue, which permitted the individual florets to remain close together. A wide variation in the number of florets per head occurred under each condition of soil moisture. Greater variability in the

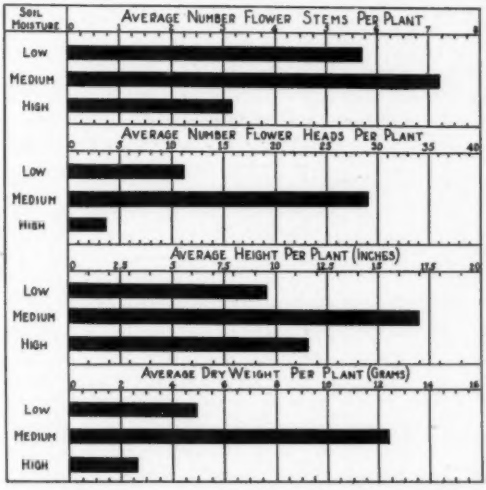

FIGURE 5.—Effect of soil moisture on the growth of red-clover plants in dune sand, Urbana, Ill., 1927 (Based on Table 3)

size of head occurred in both loam and sand when the plants were grown under extreme moisture conditions than when they were grown in soil of medium moisture.

TABLE 4.—*Effect of low, medium, and high soil moisture on the number of florets per head for five mature heads from each of two mother plants when red-clover plants were grown in Muscatine silt loam and dune sand at Urbana, Ill., 1927*

Soil type and soil-moisture condition	First growth of—			
	Flower heads	Total florets	Florets per head	Variability W*
Muscatine silt loam:				
Low	80	7,209	90±1.7	4.42
Medium	90	8,512	95±1.8	4.33
High	77	6,208	81±2.3	4.81
Dune sand:				
Low	20	1,706	85±3.4	4.52
Medium	20	1,952	98±2.5	3.94
High	19	1,406	74±4.9	5.50

* Weinberg's (7) formula of variability: $W=\sigma\frac{\sqrt{Mm-Mo}}{\sqrt{(Ma-Mo)\ (Mm-Ma)}}$

Where

σ=standard deviation.
Mm=highest class interval or highest variate.
Ma=mean.
Mo=lowest class interval or lowest variate.

Influence of Soil Moisture on Maturity of Flower Heads

When the first growth was harvested records were kept on the maturity of all flower heads, each head being classified as brown, red, or "bud." The heads were classed as brown when all the corolla tubes were brown; red, when any open floret showing color remained on the head; and bud, when the head was without any opened florets. Heads from plants growing in soil low and high in moisture matured more uniformly than those growing under favorable soil-moisture conditions. (Table 5.) This was true whether plants were grown in dune sand or in Muscatine silt loam.

Table 5.—*Effect of low, medium, and high soil moisture on the maturity of flower heads of red-clover plants when grown in Muscatine silt loam and dune sand at Urbana, Ill., 1927*

Soil type and soil-moisture condition	Number of plants	First-growth maturity of flower heads					
		Brown		Red		Bud	
		Number	Per cent	Number	Per cent	Number	Per cent
Muscatine silt loam:							
Low	45	377	72.8	92	17.8	49	9.4
Medium	45	1,057	64.4	274	16.7	311	18.9
High	43	149	87.1	9	5.3	13	7.6
Dune sand:							
Low	9	80	78.4	13	12.8	9	8.8
Medium	10	182	62.3	63	21.6	47	16.1
High	10	32	86.5	2	5.4	3	8.1

Influence of Soil Moisture on Fertilization of Red-Clover Flowers

It has been shown that a low or high soil-moisture content adversely affects the number of flowering stems, the number of heads, and the weight of dry matter produced by red-clover plants as compared to plants growing in soil of medium moisture. The difference in the number of florets produced under the various degrees of soil moisture was found to be slightly in favor of medium and low soil-moisture content. It remained to be determined whether under the conditions of the experiment there would be a difference in the number of seeds set by plants growing in soil of low, medium, or high moisture content. In the greenhouse experiments reported earlier in this paper no such difference was found.

It was hoped that plants growing in the open, like those under experiment, would be adequately pollinated by bees. From preliminary experiments conducted in 1926 it was learned that natural cross-pollination by bumblebees could not be depended upon for quantitative results in the matter of seed produced, even when colonies of bumblebees were transferred to locations adjacent to the controlled cultures.

Artificial cross-pollination, therefore, had to be resorted to, and florets were cross-pollinated between June 30 and July 20 from each of two individual plants of a different parentage growing in the same can. On most of the heads the number of florets successfully manipulated were counted, but in a few cases this was impossible on account of the condition of the florets.

From the number of seeds set, as shown in Table 6, it appears that high soil moisture is less favorable for seed setting than medium and low. It is believed, however, that the lower percentage of seed produced under high soil moisture resulted from a lack of pollen necessary for pollination rather than from a failure of the pollen or stigmatic surfaces to function. This deficiency of pollen was apparent in every case where plants were growing in soil of high moisture content. It may be assumed that the lack of pollen resulted from the unthrifty condition of the plants. Examination of the pollen from plants growing under the three moisture conditions failed to show any distinct differences in its physical characters.

TABLE 6.—*Effect of low, medium, and high soil moisture on the number of seeds set by flowers, artificially cross-pollinated, when two plants were growing in the same can in Muscatine silt loam and in dune sand at Urbana, Ill., 1927*

Soil type and soil-moisture condition	First growth				
	Number of plants	Total florets on head	Number of florets cross-pollinated	Florets setting seed	
				Number	Per cent
Muscatine silt loam:					
Low	10	968	385	263	68.3
Medium	12	1,204	468	369	78.8
High	14	1,106	475	281	59.1
Dune sand:					
Low	4	455	150	121	80.7
Medium	4	428	160	101	63.1
High	4	176	[a] 143	73	51.0

[a] Number of cross-pollinated florets on 2 heads approximated.

FIELD STUDIES

RESULTS OF POLLINATIONS MADE IN JUNE, JULY, AND AUGUST, 1927

In the previous investigations where plants were grown under controlled conditions, the degree of soil moisture and atmospheric humidity did not limit the setting of red-clover seed. In the field, many influences are operative, and it is difficult to separate and control any one factor. It was desirable, therefore, to determine how far the conclusion reached by a study of red-clover plants under control would be confirmed by a study of plants in the field.

The material available for this experiment consisted of vigorous second-year clover, growing on Muscatine silt loam in a high state of fertility. It was decided to cross-pollinate florets during the blooming season, when the combined influence of various atmospheric and soil-moisture conditions might be met.

Two red-clover plants were selected a foot apart, and 12 soil borings were made in a clockwise manner in a radius of 1 foot from the nearer plant. Moisture determinations were made from each boring of each of three zone depths, 9, 18, and 28 inches. The results of these determinations indicated that four equidistant borings represented the soil moisture of the main soil area from which water was absorbed by the roots. The borings were made, generally once or twice a week, from June 4 to September 22, and the percentage of moisture of each sample was determined. Each plant to be cross-pollinated was used as an axis from which the borings were located.

In order to obtain records of atmospheric moisture at the time the pollinations were made, a standard United States Weather Bureau instrument shelter was constructed and located at one edge of the plot. The shelter was so situated that when a hygrothermograph was placed inside, the instrument was 18 inches from the surface of the soil, a distance approximating the height of the clover heads. A sling psychrometer was used daily to check the readings of the hygrothermograph at the time of pollination.

Practically every day during the months of June, July, and August and during the first three weeks of September two plants located not more than a foot apart were selected for experimentation. Each plant had two flower heads of approximately the same degree of maturity, that is, from one-fourth to one-half in bloom. The heads were tagged and numbered, and one head from each plant was pollinated with pollen from a head on the other plant. The manipulation was similar to that used on plants in the greenhouse experiments. The greater number of the pollinated florets were manipulated three times, an effort being made to cross-pollinate all the florets on each head. The different pollinations of the same heads, however, were usually made two days apart. Pollinations were made during various hours of the day from 8 a. m. until 7 p. m. When the heads were cross-pollinated, observations were recorded as to whether the day was cloudy, partly cloudy, or clear; the time and date of the manipulation; the maturity of the heads; the physical condition of the pollen; and other notes descriptive of environmental and plant conditions. The heads were harvested four to five weeks after they had been pollinated, and notes were recorded as to the height, number of flower stems and heads, vigor, and the presence or absence of seed on open-pollinated heads of the female plant.

Because of the increase of insects which destroyed many florets and heads during the latter part of August it became necessary to bag the heads after pollination. Under these circumstances only one head with approximately 30 open florets on each plant was selected and cross-pollinated. The florets were counted and the heads covered with small cheesecloth bags until harvested. The total numbers of florets and seeds in each head were later counted in the laboratory. The hygrothermograph records were employed as a basis for the calculation of the saturation deficit, which permitted comparisons to be made of the variations of the atmospheric moisture under varying temperatures.

Weather conditions were ideal for the tests, as extreme and normal relationships prevailed at different times during the season. The plots were on low ground, and during periods of heavy rainfall water stood for several days 3 to 4 inches over the surface of the soil in the lower areas.

During the season a total of 145 heads were cross-pollinated, including heads from both first and second growth. In most cases the florets on each head were pollinated at different times, usually under a different set of environmental conditions. The uneven opening of the florets necessitated more than one pollination.

The results of the pollinations made during June, July, and August clearly indicate that moisture is not a limiting factor in the setting of red-clover seed. In many cases where the combined effect of high atmospheric and soil-moisture conditions were operative at the same

time seed set as freely as when soil and air were relatively dry. At different times during this period florets from 11 heads were pollinated when the soil was saturated or supersaturated. In all of these cases the number of seeds set was as great as under conditions of lower soil moisture. Florets pollinated when they were wet with dew or rain produced seed. The number of seeds, however, was less than from heads under dry conditions. The washing of the pollen from the needle was believed to be partly responsible for this decrease of seed setting. Likewise, the degree of atmospheric humidity did not appear to affect the setting of seed. There appeared to be no difference in the relative quantities of seed set by florets of vigorous plants and by those of weak plants or between first-growth or second-growth flowers.

RESULTS OF HAND POLLINATION, SEPTEMBER, 1927

The results of the pollinations made during September are considered representative of those of the previous months, since similar climatic conditions prevailed. A presentation of the data is possible, as a known number of florets on each head were pollinated at one time under a definite set of conditions, which necessarily must include both atmospheric and soil moisture. (Table 7.) There was one failure to obtain a setting of seed during September, and this failure appeared to result from nonfunctional pollen. The other plant involved in the cross, however, produced 20 seeds from 22 pollinated florets.

TABLE 7.—*Effect of soil moisture on the setting of seed when the florets of red-clover plants were artificially cross-pollinated under field conditions at Urbana, Ill., September, 1927*

Soil-moisture range	Second growth			
	Number of plants	Number of florets cross-pollinated	Florets setting seed	
			Number	Per cent
18.0 to 22.5 per cent	12	337	231	69
22.6 to 27.5 per cent	21	583	418	72
27.5 per cent to saturation	16	406	273	67
Supersaturation	4	94	50	53

INFLUENCE OF SOIL MOISTURE ON SEED SETTING

Florets of four plants were manipulated when the soil was supersaturated, many when the soil was nearly saturated, but the greatest number of pollinations were made when the soil moisture appeared to be most favorable for growth. Low conditions of soil moisture were experienced at only one period and this did not represent an extreme condition.

The percentage of seed set when the soil was supersaturated apparently is somewhat lower than that set under other soil-moisture conditions. This difference is not significant, it is believed, because wide variations existed in all ranges of soil moisture, and the small number of individuals in the supersaturated range undoubtedly contributed to the position it occupies. It happens that two of the four heads produced percentages of 72 and 87, which are as great as or greater than the percentage set at the other soil-moisture ranges. Also, each of the lower ranges of soil moisture have at least three individuals which set as low a percentage of seed as the other two plants under the supersaturated condition.

Influence of Meteoric Water on Pollination and Fertilization of Red-Clover Florets

During these investigations, florets of six plants were cross-pollinated when their perianths were wet with dew or rain, and if the number of seeds harvested is compared with the florets pollinated under dry conditions it is apparent that meteoric water does not limit the functioning of the pollen and the stigmatic surfaces of red-clover florets. Manipulation of the florets was difficult, as the pollen was often washed off the needle, and it was sometimes questionable whether pollen was successfully transferred.

The results presented in Table 8 show that florets wet with dew or rain at the time of pollination are capable of seed production. The percentage of florets setting seed was greater than the number normally set under field conditions. The chances of the pollen not being successfully transferred were great and possibly caused the wide variation in the number of seeds set.

Table 8.—*Effect of meteoric water on seed setting, when florets of red-clover plants were artificially cross-pollinated under field conditions while flowers were wet with dew or rain at Urbana, Ill., September, 1927*

Plant No.		Number of florets cross-pollinated	Florets setting seed	
Female	Male		Number	Per cent
100	101	21	7	33
101	100	22	20	91
102	103	26	9	35
103	102	19	10	53
120	121	26	19	73
121	120	27	18	67
Total or average		141	83	59

When the florets were tripped, the pollen was observed to be clumped together in large masses. It appeared to adhere to dry parts of the needle, but no affinity existed between the pollen and the moistened surfaces. Pollen was scarcely visible on the proboscides, legs, and body hairs of bumblebees which were caught while working the florets at the time the hand pollinations were made. During the season two heavy, precipitous rains occurred, followed by high temperatures. This condition appeared to cause a deterioration of the pollen which had been dehisced preceding the rain. A few hours later, however, pollen from newly opened florets functioned when used in cross-pollination.

Atmospheric Humidity as Related to Seed Setting

Atmospheric humidity during September varied widely. The results from pollinating florets under these conditions are presented in Table 9. During this period, the florets were pollinated under atmospheric conditions such that the saturation deficit varied from 0.109 to 0.654. In a few cases, rain was falling at the time of manipulation, the florets being shielded so that the rain did not touch the floret or needle.

TABLE 9.—*Effect of atmospheric humidity on the production of red-clover seed under field conditions, when florets were artificially cross-pollinated at Urbana, Ill., September, 1927*

Saturation deficit range	Number of plants	Number of florets cross-pollinated	Florets setting seed	
			Number	Per cent
0 to 0.199	8	219	131	60
0.200 to 0.299	18	497	352	71
0.300 to 0.399	10	279	188	67
0.400 to 0.499	2	51	32	63
0.500 to 0.599	9	228	172	75
0.600 to 0.699	6	146	97	66

The results of these investigations agreed with those conducted in the greenhouse in that atmospheric humidity was not a limiting factor in the production of red-clover seed. Although it might appear from the data in Table 9 that a low saturation deficit was not so favorable for the setting of seed as a drier atmosphere, most of the heads worked on at a deficit ranging from 0 to 0.199 were pollinated when the florets were wet with meteoric water, which increased the chances of unsuccessful pollination. The hygrothermograph records showed that during practically every night the atmospheric humidity increased approximately to saturation.

DISCUSSION

The foregoing results show that high atmospheric moisture in both field and greenhouse studies did not limit the setting of red-clover seed. If, as Martin found, artificial germination of pollen is delicately adjusted to a water supply, the stigmatic surface must be able to regulate the water which it furnished in accordance with the atmospheric moisture present. As the atmospheric humidity often varies quickly and widely, the regulation of the water produced by the stigmatic surface must be rapid, and it seems improbable that this is the case. In Martin's investigations on artificial germination it appears more likely that factors other than humidity were operative which were not studied. The atmospheric moisture surrounding the pollen grain at germination is believed to be of little consequence in its functioning. This conclusion is further substantiated by the results of the investigations on the effect of meteoric water on pollination and fertilization of the florets. Seed was produced in the field when red-clover florets wet with meteoric water were pollinated. In these experiments the stigma had no control over the quantity of water that came in contact with the germinating pollen.

Soil moisture whether high or low does not prevent seed setting, although plants grown constantly in wet soil may set a smaller percentage of seed than those in a dry or medium-wet soil. This appears to be due not to the influence of moisture on the functions of pollen or stigma, but to the fact that the plant, being in an unhealthy condition, fails to produce sufficient pollen. Medium soil moisture is conducive to vegetative growth and the production of flowers, which if properly pollinated would of course produce the largest yield of seed. The question arises, however, whether plants grown in soil in which the moisture is slightly below the optimum for growth

would not by maturing their flowers more uniformly, be more satisfactory for seed production. While infertile ovules may occur, they are apparently not caused by moisture conditions and are not present to a greater extent in the first than in the second growth.

Although these investigations indicate that variations in moisture conditions surrounding the plant at the time of reproduction do not limit seed setting, it is believed that moisture indirectly affects red-clover seed production in that it appears to influence the activities of pollinating insects. Microscopic examination of bumblebees after visiting flowers wet with meteoric water showed an absence of pollen on their body hairs and proboscides. Either the pollen did not adhere after being moistened or it was washed off the bees by excess water. During the seasons of 1926 and 1927 it was observed that more bees were engaged in visiting flower heads between noon and 5 p. m. than in the morning. Hygrothermograph records showed that generally the largest saturation deficit occurred at the time of the greatest activity of the bees.

Red-clover pollen is often considered as being dry when dehisced from the anthers. Observations made under a wide range of environmental conditions indicate that pollen is always somewhat sticky and moist. Hence, red-clover plants may be classified as entomophilous. During periods of small saturation deficits pollen did not fly as freely as when a large deficit occurred. When the florets were wet with meteoric water, the pollen was generally found in relatively large sticky amorphous masses. In a few cases, however, pollen was discharged when the florets were tripped.

When a mature floret is tripped, the sudden release of the filaments and anthers from the carina discharges a part of the pollen. A large proportion remains on the anthers until the pollen comes in contact with outside agencies. When the floret first opens, the pollen is usually in lumpy, amorphous masses and very seldom is any discharged when the floret is tripped, but as the open flower advances in maturity a greater quantity is ejected. During these investigations observations have shown that, in visiting flower heads, the bumblebee has a preference for those in full bloom and those in which the corolla tissue has become brown. At this stage it is possible that many of the ovules have disintegrated and the quantity of viable pollen is not so abundant as in florets less advanced in maturity. Considerable variation in the quantity of pollen which is ejected exists between different florets of the same head. The greater part of this variation, no doubt, results from a difference in the maturity of the flowers. In several cases florets have been examined in which the pollen appeared never to have been functional.

SUMMARY

This study concerns the relation of atmospheric and soil moisture to seed production of red clover.

Soil moisture, whether low, medium, or high, did not prevent the setting of red-clover seed either when plants were grown in the field or under controlled conditions.

Medium soil-moisture content when compared to low or high soil-moisture content increased the productiveness of red-clover plants with respect to both the number of heads and flower stems and vege-

tative growth. Plants grown in soil low in moisture produced a greater number of heads and more vegetative growth than those grown in soil high in moisture.

Low soil moisture and high soil moisture were more conducive to early flowering and uniform maturity than a medium soil moisture.

A greater number of florets per head were produced when plants were grown in soils of medium and low moisture content than when they were grown in soils of high moisture content. No significant difference was observed in the number of florets per head on plants grown in soils of medium moisture and those grown in soils of low moisture content.

The differences in soil moisture did not appear to affect the physical condition of the pollen.

Atmospheric humidity did not affect the setting of red-clover seed under greenhouse or field conditions. Pollen under conditions of high atmospheric humidity showed a greater tendency to cohere than under conditions of low humidity.

When the plants were growing under field conditions, cross-pollinated red-clover florets wet with meteoric water produced seed.

In laboratory tests not all pollen grains burst immediately when placed in water.

LITERATURE CITED

(1) Hansgirg, A.
1897. Beiträge zur biologie u. morphologie des pollens. Sitzber. k. Böhm Gesell. Wiss. 23, 76 p.

(2) Hilgard, E. W.
1911. Soils. their formation, properties, composition, and relations to climate and plant growth in the humid and arid regions. 593 p., illus. New York and London.

(3) Lidforss, B.
1896. Zur biologie des pollens. Jahrb. Wiss. Bot. 29: 1–38.

(4) ———
1899. Weitere beiträge zur biologie des pollens. Jahrb. Wiss. Bot. 33: 232–312.

(5) Martin, J. N.
1913. The physiology of the pollen of trifolium pratense. Bot. Gaz. 56: 112–126, illus.

(6) Schlecht, F.
1921. Untersuchungen über die befruchtungsverhältnisse bei rotklee (trifolium pratense). Ztschr. Pflanzenzucht. 8: 121–157, illus.

(7) Weinberg, W.
1926. Zur messung der streuung bei extensiven masszahlen. Arch. Rassen u. Gesell. Biol. 18: 84.

(8) Welton, F. A., and Morris, V. H.
1925. Climate and the clover crop. Jour. Amer. Soc. Agron. 17: 790–800, illus.

(9) Westgate, J. M., and Coe, H. S., in collaboration with Wiancko, A. T., Robbins, F. E., Hughes, H. D., Pammel, L. H., and Martin, J. N.
1915. Red-clover seed production: pollination studies. U. S. Dept. Agr. Bul. 289, 31 p., illus.

(10) Williams, R. D.
1925. Studies concerning the pollination, fertilization, and breeding of red clover. Univ. Col. Wales, Aberystwyth, Welsh Plant Breeding Sta. Ser. H (4). Seasons 1921–1924, 58 p., illus.

AERIAL CROWN GALL OF THE APPLE[1]

By E. A. SIEGLER, *Associate Pathologist*, and R. B. PIPER, *Assistant Scientific Aide*, *Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Malformations of the apple variously termed "aerial crown galls," "stem tumors," and "burr knots" have been well known for many years. Knight (*9*)[2] in 1809 referred to them in connection with their use in propagating affected varieties by cuttings. Darwin (*2, v. 2, p. 39–40*), evidently referred to them when he wrote: "In Chiloe [an island off the coast of Chile] the inhabitants possess a marvelously short method of making an orchard. At the lower part of almost every branch, small, conical, brown, wrinkled points project; these are always ready to change into roots, as may sometimes be seen, where any mud has been accidentally splashed against the tree." He then described how branches from these trees are buried in the soil in order to form shoots. Garman (*4*) illustrated typical galls and referred to them as a knot disease, probably due to a fungus. Hedgcock (*6*) described these swellings as a form of crown gall, basing his assumption on the fact that they rooted and formed the typical woolly-knot type of crown gall when buried in the soil. He also specifically stated that cuttings from these malformations readily threw out roots when placed in moist sand or soil, and he called attention to the fact that these swellings "develop, internally, incipient adventitious roots, which * * * break forth, * * * forming a warty knot." Smith, Brown, and Townsend (*15*) also believed these malformations to be a form of crown gall. Despite the fact that the possibilities of vegetative propagation by use of these malformations were apparently well known, this method is believed never to have been put into actual use in the United States, probably because of the belief that it would not be practicable.

In the literature there is known to the writers only one reference[3] in which isolation of the crown-gall organism (*Bacterium tumefaciens* Sm. and Town.) has been reported. Taubenhaus reported this isolation but failed to state whether he tested the validity of the organisms he isolated. This test is a necessary safeguard against wrong interpretations.

Brown (*1*), in reporting experiments that extended over a long period, stated her inability to isolate the crown-gall organism from typical stem tumors. She also noted that while plant pathologists and entomologists in general have referred to this type of overgrowth as a manifestation of crown gall, this assumption was based on rather

[1] Received for publication Feb. 2, 1929; issued August, 1929.

[2] Reference is made by number (italic) to "Literature cited," p. 261.

[3] ADAMS, J. F. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul. Sup. 28: 308. 1923. [Mimeographed.]

inconclusive evidence. Swingle (*16*) in view of this statement and as a result of observations concluded that these aerial galls were merely varietal responses and urged the further consideration of propagating these affected varieties by means of cuttings from these galls. Melhus (*10*) and several other investigators apparently agreed with this diagnosis. Muncie and Shippy (*11*) also reported negative results in isolations from burr knots of apple, but noted that certain environmental conditions favored the production of burr knots.

Siegler (*13*) isolated, from malformations on apple roots, an organism which appeared identical with the apple strain of *Bacterium tumefaciens*. This organism was tested on apple shoots, as well as on certain other hosts, in order to determine its pathogenicity. It was noted that inoculations with this organism on apple shoots, growing under greenhouse conditions, resulted in malformations which resembled typical aerial galls.

EXPERIMENTS

The experiments reported in this paper were undertaken mainly for the purpose of testing the pathogenicity of certain bacterial organisms isolated from malformations on grafted apple trees. It was soon ascertained that the strain of the crown-gall organism that appeared identical with the daisy strain of Smith et al. (*15*) failed, with rare exceptions, to cause infection when inoculated on tender shoots of the apple, whereas a certain other strain, which may be called the apple strain, readily caused infection. This is the strain referred to by Siegler (*13*) as being probably identical with the apple strain of Smith et al. (*15*) and as being pathogenic on certain hosts, including the apple. It is thought that this is the same organism that Riker et al. (*12*) classed as one of their nonpathogenic strains.

Inoculations were made mostly by means of needle punctures, either single or grouped. Hypodermic needles and scalpels were also employed. The hypodermic needle was a standard 24-gauge make, which at the suggestion of M. B. Waite was slightly modified to prevent clogging. The inoculum was placed at various depths in the tissues. In some cases the hypodermic needle by accident was forced entirely through the tissues so that it projected from the opposite side. Control punctures were always made in the same manner with the same type of instrument either on the opposite side of the same twigs or on twigs which would correspond to the inoculated ones so far as growth characteristics were concerned. Inoculations were generally made on the growth of the current year, but they were also made on limbs up to 6 years of age and 1 inch in diameter.

In most of the experiments young seedlings grown from seeds of varieties susceptible to the aerial form of crown gall were used. The seeds were mostly from open-pollinated fruits of the Chenango and Buckskin varieties. Inoculations were also made on 10-year-old Early Ripe and Givens trees. The details of the inoculation experiments are listed in Table 1.

TABLE 1.—*Results of inoculating apple stems with apple and peach strains of crown gall*

[In the eighth column the numerator of the fraction indicates the number of shoots inoculated and the denominator the number of punctures]

Experiment	Host		Date of inoculation	Duration of experiment (days)	Source of inoculum	Method of inoculation	Shoots and punctures	Shoots infected		Punctures infected	
	Variety	Age						Number	Per cent	Number	Per cent
No. 62-G	Northern Spy seedling	4 months	June 13, 1927	34	Apple strain (474-B-1)	Hypodermic needle punctures.	1/30	1	100	5	16.6
Control G	do	do	do	34	Sterile water	do	1/30	0	0	0	0
No. 66-L	do	do	do	34	Apple strain (474-B-1) in water suspension.	do	1/25	1	100	6	24
Control L	do	do	do	34	Sterile water	do	1/25	0	0	0	0
No. 64-A	do	do	Aug. 15, 1927	60	Apple strain (474-B-1)	Single needle punctures	2/30	2	100	7	23.3
Control A	do	do	do	60	Sterile needle	do	2/30	0	0	0	0
No. 64-B	do	do	do	60	Apple strain (486-101)	do	2/30	2	100	10	33.3
Control B	do	do	do	60	Sterile needle	do	2/30	0	0	0	0
No. 64-C	do	do	do	60	Peach strain	do	2/30	0	0	0	0
Control C	do	do	do	60	Sterile needle	do	2/30	0	0	0	0
No. 85-B	Early Ripe	10 years	Nov. 11, 1927	300	Apple strain (455-9-A-9)	do	1/25	1	100	2	8
Control B	do	do	do	300	Sterile needle	do	2/50	0	0	0	0
No. 85-D	do	do	do	300	Apple strain (474-B-1)	do	1/25	1	100	1	4
Control D	do	do	do	300	Sterile needle	do	2/50	0	0	0	0
No. 85-G	do	do	Nov. 2, 1927	300	Peach strain	do	1/25	0	0	0	0
Control G	do	do	do	300	Sterile needle	do	1/25	0	0	0	0
No. 114-B	Chenango seedling	1 year	Feb. 24, 1928	211	Apple strain (486-101)	do	5/100	5	100	7	7
Control B	do	do	do	211	Sterile needle	do	5/100	0	0	0	0
No. 114-C	do	do	do	211	Apple strain (487-6)	do	5/100	3	60	3	3
Control C	do	do	do	211	Sterile needle	do	5/100	0	0	0	0
No. 114-D	do	do	do	211	Apple strain (510-2)	do	5/100	4	80	9	9
Control D	do	do	do	211	Sterile needle	do	5/100	0	0	0	0
No. 114-E	do	do	do	211	Peach strain	do	5/100	0	0	0	0
Control E	do	do	do	211	Sterile needle	do	5/100	0	0	0	0
No. 116-A	do	do	Feb. 29, 1928	206	Apple strain (486-1)	do	20/300	14	70	25	8.3
Control A	do	do	do	206	Sterile needle	do	20/300	0	0	0	0
No. 116-B	do	do	do	206	Peach strain	do	20/300	1	5	1	.33
Control B	do	do	do	206	Sterile needle	do	20/300	0	0	0	0
No. 120	do	do	Mar. 1, 1928	205	Apple strain (486 and 505)	do	5/50	3	60	3	6
Control	do	do	do	205	Sterile needle	do	5/50	0	0	0	0
No. 138	Buckskin seedling	4 months	May 7, 1928	149	Apple strain (486-101)	Very shallow single needle punctures.	6/60	1	16.6	1	1.6
Control	do	do	do	149	Sterile needle	do	6/60	0	0	0	0
No. 140	Chenango seedling	do	May 9, 1928	136	Apple strain (486-101)	Grouped punctures of 5 made with fine needle.	1/6	1	100	4	66.6
Control	do	do	do	136	Sterile needle	do	1/6	0	0	0	0
No. 143	do	do	do	136	Apple strain (486-101)	Single needle punctures	3/30	2	66.6	7	23.3

TABLE 1.—*Results of inoculating apple stems with apple and peach strains of crown gall*—Continued

Experiment	Host		Date of inoculation	Duration of experiment (days)	Source of inoculum	Method of inoculation	Shoots and punctures	Shoots infected		Punctures infected	
	Variety	Age						Number	Per cent	Number	Per cent
Control	Chenango seedling	4 months	May 9, 1928	136	Sterile needle	Single needle punctures	3/30	0	0	0	0
No. 146-B	Early ripe	10 years	May 11, 1928	122	Apple strain (486-101) in water suspension.	Hypodermic needle punctures.	5/75	3	60	9	12
Control B	do	do	do	122	Sterile water	do	5/75	0	0	0	0
No. 146-D	do	do	do	122	Apple strain (486-101) in water suspension.	do	5/75	5	100	11	15
Control D	do	do	do	122	Sterile water	do	5/75	0	0	0	0
No. 150	do	do	May 18, 1928	115	Apple strain (486-101)	Single needle punctures	5/50	2	40	2	4
Control	do	do	do	115	Sterile needle	do	5/50	0	0	0	0
No. 151	do	do	May 22, 1928	111	Apple strain (486-101) in water suspension.	Hypodermic needle punctures.	16/320	16	100	58	18
Control	do	do	do	111	Sterile water	do	9/180	0	0	0	0
No. 153	Givens	do	May 25, 1928	108	Apple strain (486-101) in water suspension.	do	8/160	8	100	20	12.5
Control	do	do	do	108	Sterile water	do	5/100	0	0	0	0
No. 159	Early ripe	do	July 11, 1928	43	Apple strain (486-101)	1-year old shoot girdled at base with scalpel.	1/1	1	100	1	100
Control	do	do	do	43	Sterile scalpel	do	1/1	0	0	0	0
No. 160	do	do	July 14, 1928	58	Apple strain (486-101) in water suspension.	Hypodermic needle punctures in leaf axil under bud.	7/164	6	86	21	13
Control	do	do	do	58	Sterile water	do	4/109	0	0	0	0
No. 162	do	do	July 16, 1928	56	Apple strain (486-101)	Smeared over ½-inch cut area on 2-inch limb and punctured in.	1/1	1	100	1	100
Control	do	do	do	56	Sterile needle	Sterile needle punctures	1/1	0	0	0	0
No. 166	do	do	July 27, 1928	45	Apple strain (486-101)	Grouped needle punctures of 5.	2/10	1	50	5	50
Control	do	do	do	45	Sterile needle	do	2/10	0	0	0	0
No. 170	do	do	do	45	Apple strain (486-101)	do	1/6	1	100	5	83.3
Control	do	do	do	45	Sterile needle	do	1/6	0	0	0	0
No. 173	do	do	Aug. 7, 1928	34	Apple strain (486-101)	1-year old shoots girdled at at base with scalpel.	10/10	4	40	4	40
Control	do	do	do	34	Sterile scalpel	do	10/10	0	0	0	0

In an examination of this list of experiments it will be seen that infections were obtained consistently, but that a comparatively small percentage of infection actually resulted, when every puncture is considered. There were very few cases in which an inoculated twig failed to become infected in at least one of the punctures. The reactions obtained on apple shoots of various ages resemble in gross morphological respects the typical naturally occurring burr knots. (Fig. 1, A, B.) In their incipiency many are indistinguishable macroscopically from the small swellings usually found just above the bud and generally considered to be aphis injury. (Fig. 1, C.) The organism has been recovered from the areas designated by arrows in this figure. These small swellings may enlarge quite rapidly until they consist of a mass of roots projecting through the epidermis. (Fig. 1, D.) When the swellings have reached this stage it has rarely been possible to reisolate the organism from them. In no case have adventitious shoots appeared; in these experiments swellings made up of root primordia invariably have resulted from infection. In no case have the control punctures become infected, and they have never shown any reaction other than the slight disturbance of the tissues which normally would be expected. In cases where punctures have been relatively small the wounds have practically healed over, as shown in Figure 1, E, whereas many of the inoculated areas when not actually resulting in apparent infection still show a greater disturbance of the tissues as shown in Figure 1, F. A shoot in one of the experiments representing the highest percentage of infection is shown in Figure 2, A, with the control in Figure 2, B. It is seen that these are macroscopically indistinguishable from the naturally occurring galls. Among the infections which were secured by means of inoculations there were comparatively few cases where there appeared an outgrowth consisting of a single root rudiment as illustrated in Figure 3, A. Reisolations have been made from these infections.

On older wood, as a result of a single experiment, the chances of securing infection are apparently greater. Figure 2, C, shows swellings produced at five out of six points of inoculation by means of needle punctures in groups of five. The control punctures, made on a near-by branch, have healed over.

Rather striking examples of excessive root formation resulting from inoculations are shown in Figure 3, B, C. It is probable that the excessive root growth was favored by moisture conditions due to the closeness of these inoculated areas to the soil.

The experiments in which shoots of the current year were girdled at the base by a scalpel smeared with the inoculum proved interesting in that root primordia appeared on these girdled areas. This result may account for the presence of aerial galls on pruning wounds. Additional evidence on this point is furnished by an experiment in which the inoculum was placed on a freshly made pruning wound by means of a scalpel. One large swelling, one-eighth inch in diameter, resulted in the cortical region on the inoculated wound which was approximately one-half inch in diameter. This experiment has been repeated with like results. The control wound remained free of any abnormal growth. The root primordia which developed on the girdled twig in experiment No. 159 are shown in Figure 2, D, with the control girdled region completely healed as shown in Figure 2, E.

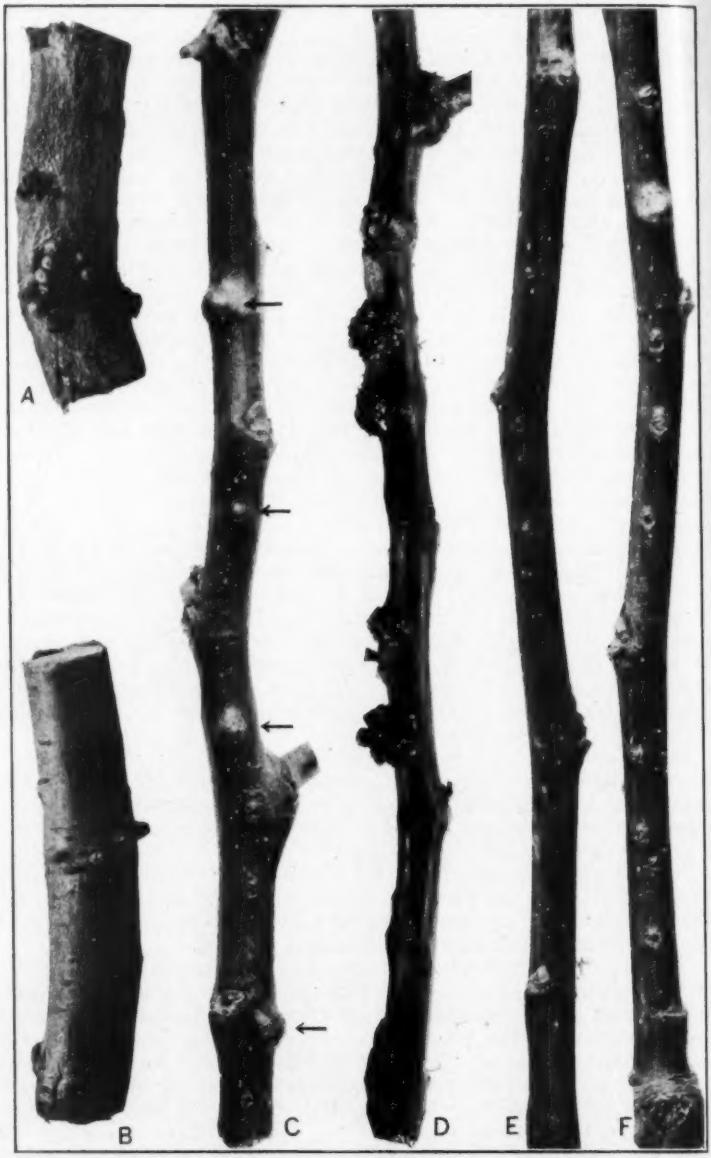

FIGURE 1.—A and B, Early stages of naturally occurring aerial galls on Early Harvest apple; C, smooth swellings which appear indistinguishable from woolly-aphis injury but which were induced by inoculation with the crown-gall organism on Early Ripe apple; time, 55 days; D, later stages of galls which were produced by artificial inoculations on a Northern Spy seedling and which show projecting roots; time, 400 days; E, control punctures on Early Ripe apple practically healed over; time, 155 days; F, shoot of Early Ripe apple on which only one puncture resulted in infection and on which the other punctures did not heal as they did in the control. All natural size

FIGURE 2.—A, Shoot showing a comparatively high percentage of galls resulting from artificial inoculations; time, 150 days; B, control punctures, which are healing over, for shoot shown in A; time, 150 days; C, infections from inoculations on older wood; time, 90 days; × ¾; D, root primordia formed in area girdled and inoculated by means of a scalpel in experiment No. 159; time, 43 days; E, control area healing after girdling with a scalpel; time, 43 days. All, Early Ripe variety. All except C natural size

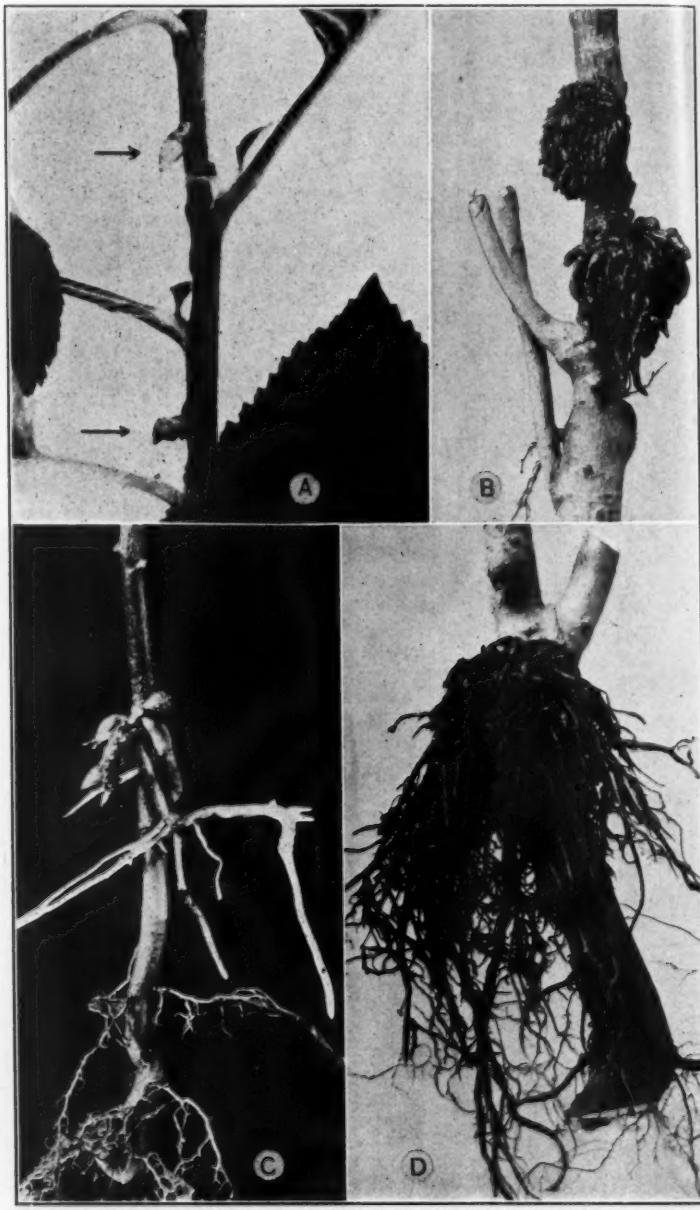

FIGURE 3.—A, Twig of a Northern Spy seedling showing single fleshy-root rudiments produced by artificial inoculations; time, 60 days; B and C, excessive fleshy root formation as a result of artificial inoculations on Chenango seedlings; time, 150 and 90 days, respectively; D, typical woolly-knot type of crown gall formed on Chenango seedling when aerial galls similar to those illustrated in Figure 1, D are planted in the soil; time, 206 days. All natural size

That these artificially produced aerial galls developed into the woolly-knot type of crown gall when planted in soil is shown in Figure 3, D. Numerous experiments repeatedly demonstrated this fact. The controls remained free of infection.

Attempts have been made to reisolate the organism from artificially produced galls of different ages. With but one exception it has been impossible to isolate the organism from galls over 65 days old. From galls less than 65 days old the organism has been reisolated only fairly consistently. In all there have been 16 attempts at reisolating the organism from artificially produced galls, and 31 per cent were successful. The reisolations have always been tested on susceptible hosts. During the last eight years numerous unsuccessful attempts have been made to isolate the crown-gall organism from naturally occurring aerial galls. In addition to these attempted isolations, macerations from galled areas have been made and the suspension inoculated into Bryophyllum. No infections were obtained on this host. All stages of natural galls have been used in these attempts at isolation.

DISCUSSION

Swingle (*16*) noted the absence in American literature of references to nonpathological, dormant, stem-borne roots in any plant. If this statement is applied to the apple and specifically to burr knots it is easily understood. Because of their resemblance to the underground form of crown gall, as stated previously, these malformations have been considered by pathologists as being of pathogenic origin. Previous to Swingle's (*16*) article, Brown (*1*) had reported her failure to obtain the crown-gall organism from the burr knots she described. Furthermore, before Hedgcock's (*6*) publication wherein he noted that these stem tumors developed incipient roots, the fact that these swellings consisted of root primordia had been well recognized. This recognition is true of Hedgcock's (*6*) statement that cuttings readily root, "developing directly into the woolly-knot form of the disease" (crown gall). It is not deemed necessary to enter into a further discussion to prove that aerial galls or burr knots have long since been recognized as root primordia and capable of readily propagating themselves by rooting when placed in the soil. That the terms "burr knot," "aerial galls," and "stem tumors" are synonymous must be taken for granted, since no one has ever explained how to differentiate them. Since it was generally accepted in this country that these aerial crown galls were of a pathogenic nature, pathologists may have been loath to suggest this means of propagation by cuttings.

From the writers' experiments it is evident that the so-called apple strain of *Bacterium tumefaciens* is capable of causing malformations morphologically identical with burr knots or stem tumors. Reisolations from relatively young infections are possible. The causal organism has been reisolated from one gall 106 days after inoculation, but the possibility that the original inoculum may have been present should not be overlooked. It has not been possible to culture the organism from older artificial infections or from naturally occurring galls of any age. It may be permissible to reason that the organism survives but a short time in the artifically produced galls. It may

also die very readily in the natural galls. Smith et al. (*15*), in discussing the loss of virulence in culture of these crown-gall organisms, raised the following questions: "Why should not virulence often disappear from organisms buried inside the tissues of tumors? And is not the fact that the tumor has ceased to be active and the host has gained the ascendency evidence of this?" Israilsky (*7*) noted the disappearance of the organism from tumors and considered it due to the presence of a bacteriophage. Kauffman (*8*), using inoculum isolated from a mouse, also recorded failures to reisolate the organism from fully developed sunflower tumors, and concluded that after initial pathological growth has started the subsequent growth may be autonomous. This explanation may serve to account for the development of aerial galls after the initial stimulus has been supplied and definite root tissues have been differentiated. There is no normal way for these partially developed roots to function. They remain in a state of quiescence, although upon being placed in the soil under normal environmental conditions they become capable of functioning, at least to a certain extent. It is quite possible that the crown-gall organism may find this semidormant condition of the tissue uncongenial and the organism may die out because of this condition alone. Smith et al. (*15*), as noted above, adopted a similar line of reasoning when, in referring to the dying out of organisms and malformations produced by hyperplasia resulting from inoculations with the daisy strain, they noted the loss of virulence. The writers' viewpoint is that in these aerial tumors there is not a hyperplasia but an organization similar to a teratoma; that is, there are normal tissues occurring in a region where they are not normally found. They are apparently able to exist, but they may not offer conditions favorable to the growth of the organism. It should, therefore, be considered just as remarkable if the organisms lived in this tissue as if they either became attenuated or died.

That there are tissues in the stem of the apple capable of forming roots is generally recognized. The ease with which some varieties root from the scion part of the graft is evidence of this. Furthermore, certain varieties of pear and other fruits are propagated from cuttings which root quite readily under certain conditions. Just what stimulus is required for roots to be formed in an environment not normally favorable to their development is not known. The writers' experiments have proved that the introduction of the crown-gall organism into the stem tissues is at least one stimulus which causes the formation of root primordia. In regard to the failure to isolate the organism from naturally occurring galls there are many examples of other diseases from which the causal organism can not always be isolated. For example, the pear-blight bacillus is exceedingly easy to demonstrate in tissues at certain stages of infection, but most infections quickly die out.

The fact that burr knots are found more commonly on certain varieties does not necessarily make them a varietal characteristic as Swingle (*16*) argued. They may just as readily be regarded as evidences of varietal susceptibility. Nor does the occurrence of these tumors on vigorous trees prove or disprove their pathogenic nature. The writers' observations, however, are that, in general, mature trees which show numerous galls are rather weak. At the Arlington Experiment Farm the varieties chiefly affected are of no commercial

importance. While no attempt has been made to make a survey of this disease, it is known to occur generally throughout the world. In the writers' experience the Early Harvest appears to be the most important commercial variety on which galls are most frequently encountered. In 1918 at Jeff, Ala., every Early Harvest tree in an orchard of approximately 20 acres of Early Harvest and Yellow Transparent varieties was severely affected with burr knots. The trees were seriously weakened because the galls had almost girdled the main limbs and crotches. When one notes the great number of galls that may be present (fig. 4, A), and examines the areas surrounding the older burr knots, it is difficult to understand how they can be deemed uninjurious. The mechanical girdling effect in itself would appear to suffice, regardless of the means of entrance of decay organisms and the susceptibility of the affected areas to other injury as illustrated in Figure 4, B.

Hatton, Wormald, and Witt (*5*), after unsuccessful attempts to isolate an organism from burr knots, and as a result of other experiments, concluded that they are of a nonpathogenic nature. Doidge (*3*), however, noted the rapid spread of aerial galls on various hosts and evidently did not question the pathogenic nature of the galls. She noted that galls on the limbs and twigs usually originated at some point where an external injury had been caused by hail or other means.

The woolly apple aphid is capable of causing swellings on the roots of apples. These aphid galls are quite distinct from crown gall. This insect also spends part of its life on the woody tissues aboveground, and it is frequently found, in groups, in the regions of the leaf axils. Swellings form in those regions and elsewhere apparently as a result of the aphid punctures. Such swellings follow the feeding of aphid colonies on various parts of the tree. While there is no proof that aphids or other insects are agents in initiating and spreading aerial crown gall, observations and knowledge of the life history of this insect would suggest that this is quite possible. It seems probable, as Brown (*1*) suggested, that aphids could carry an inoculation into the punctures they make. It should be noted, however, that aphids have never been proved to be agents in transmitting the crown-gall organism to the roots. In the writers' experiments infection has seldom been secured by injecting the inoculum into the roots by means of needle punctures, while infection is more readily obtained on the shoots of the current year by inoculations with a needle of the same size. This difficulty in securing artificial infection in the roots by means of a puncture with a small needle may possibly account for the belief that aphids do not initiate and spread the crown-gall disease in the roots. A further discussion of the rôle they may play in connection with aerial crown gall is not within the scope of this paper.

That intumescences are produced on many hosts, including the apple, by means of chemical stimulation seems well established. Much experimental evidence is given by Smith, especially in his recent publication (*14*), wherein he concluded that "many of the common teratological forms in plants and animals are due to parasitic or mechanical traumatic displacements occurring in early life." Wallace (*17*) reported the production of intumescences on twigs on the Yellow Transparent variety by exposure to ethylene gas. These intumescences do not resemble aerial crown gall in that there is no

FIGURE 4.—A, Branches of an apple tree showing numerous galls; B, injurious effect of a gall to the area surrounding it. Both of the Rasmussen variety and greatly reduced

evidence of the root rudiments. This variety, however, is not considered susceptible to aerial crown gall.

The malformations obtained by Smith et al. (*15*) and by Brown (*1*) on apple shoots by inoculations with the peach strain of the crown-gall organism are quite distinct from the ones the writers have secured by means of inoculations with the apple strain. While root formations may be found in the galls illustrated by Smith et al. (*15*) and by Brown (*1*), it is evident that the swellings are a result of either hypertrophy or hyperplasia. The writers have rarely been able to secure infections on the apple by aboveground inoculations with the so-called peach or daisy strain, but this strain readily causes infection on the stems of peach and hosts other than the apple.

Smith et al. (*15*) noted differences in the strains of the crown-gall organism, but the fact that these differences have apparently not been generally recognized is probably the reason why inoculations similar to the ones described here have not been made previously.

SUMMARY

Malformations which are morphologically identical with those known as aerial crown galls, stem tumors, or burr knots have been produced consistently on the apple by means of inoculations with the apple strain of *Bacterium tumefaciens*.

The organism has been reisolated from these galls only within comparatively short periods after infection. It has not been isolated from naturally occurring aerial galls.

The evidence given here lends some support to the hypothesis that the natural occurrence of these malformations is due to infections with the apple strain of the crown-gall organism. It does not preclude the possibility that other agencies or factors may cause these malformations.

LITERATURE CITED

(1) Brown, N. A.
1924. An apple stem-tumor not crown gall. Jour. Agr. Research 27: 695–698, illus.

(2) Darwin, C. R.
1846. Journal of researches into the natural history and geology of the countries visited during the voyage of H. M. S. Beagle round the world, under the command of Capt. Fitz Roy, R. N. 2 v., illus. New York.

(3) Doidge, E. M.
1921. Crown-gall. Bacterium tumefaciens—Smith and Townsend. Jour. Dept. Agr. So. Africa 3: 64–67, illus.

(4) Garman, H.
1901. I. Diseases of nursery stock, with a list of inspected Kentucky nurseries. Ky. Agr. Expt. Sta. Bul. 93: [101]–111, illus.

(5) Hatton, R. G., Wormald, H., and Witt, A. W.
1926. On "burr-knots" of fruit trees. Jour. Pomol. and Hort. Sci. 5: 195–204, illus.

(6) Hedgcock, G. G.
1910. Field studies of the crown-gall and hairy-root of the apple tree. U. S. Dept. Agr., Bur. Plant Indus. Bul. 186, 108 p., illus.

(7) Israilsky, W. P. (Izrailsky, V. P.)
1927. Bakteriophage und pflanzenkrebs. Centbl. Bakt. [etc. (II) 71: 302–311.

51541—29——3

(8) KAUFFMANN, F.
1927. WEITERE ERFAHRUNGEN MIT TUMEFACIENS-STÄMMEN. Ztschr. Krebsforsch. 26: [18]–20.
(9) KNIGHT, T. A.
1809. ON THE ORIGIN AND FORMATION OF ROOTS. Roy. Soc. [London] Phil. Trans. 99: 169–176.
(10) MELHUS, I. E.
1926. CROWN GALL OF APPLE NURSERY STOCK. Jour. Econ. Ent. 19: 356–365.
(11) MUNCIE, J. H., and SHIPPY, W. B.
1928. OVERGROWTHS AND HAIRY ROOT ON NURSERY APPLE AND QUINCE TREES. (Abstract) Phytopathology 18: 127.
(12) RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., and KEITT, G. W.
1928. THE RELATION OF CERTAIN BACTERIA TO THE DEVELOPMENT OF ROOTS. Science (n. s.) 68: 357–359.
(13) SIEGLER, E. A.
1928. STUDIES ON THE ETIOLOGY OF APPLE CROWN GALL. Jour. Agr. Research 37: 301–313, illus.
(14) SMITH, E. F.
1927. TUMORS, CYSTS, PITH-BUNDLES, AND FLORAL PROLIFERATIONS IN HELIANTHUS. Mem. Nat. Acad. Sci. 22(4), 51 p., illus.
(15) ——— BROWN, N. A., and TOWNSEND, C. O.
1911. CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY. U. S. Dept. Agr., Bur. Plant Indus. Bul. 213, 215 p., illus.
(16) SWINGLE, C. F.
1925. BURR-KNOT OF APPLE TREES. ITS RELATION TO CROWNGALL AND TO VEGETATIVE PROPAGATION. Jour. Heredity 16: 313–320, illus.
(17) WALLACE, R. H.
1927. THE PRODUCTION OF INTUMESCENCES IN TRANSPARENT APPLE BY ETHYLENE GAS AS AFFECTED BY EXTERNAL AND INTERNAL CONDITIONS. Bul. Torrey Bot. Club 54: 499–542, illus.

SOME FACTORS INVOLVED IN THE WINTERKILLING OF ALFALFA[1]

By J. L. WEIMER[2]

Senior Pathologist, Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The importance of winterkilling in relation to agriculture is attested by the extensive literature on the subject which has accumulated over a period of years. Many types of freezing injury have been studied on a large number of different plants and from many points of view. Nevertheless, there still remains much to be learned before a comprehensive knowledge of all phases of this subject is obtained. It is the purpose of the present paper to present the data accumulated during the last two years in greenhouse and laboratory studies dealing with the freezing and killing points of alfalfa root tissue and some factors influencing them. It is recognized by the writer that some of the experiments, which have not been carried out on such a large scale or repeated so often as desirable, are inconclusive. The only reasons for presenting the results of such experiments at this time are the impracticability of continuing this work and the desire to make these data available to others interested in the subject.

As this paper deals with only a limited phase of winter injury no attempt has been made to give an exhaustive survey of the literature on this subject. Such reviews have been made by Chandler (*4*),[3] Raos (*18*), Abbe (*1*), Wiegand (*21*), Blackman (*3*), Hildreth (*9*), Newton (*16*), Martin (*14*), and others. Steinmetz (*19*) has recently reviewed the literature on the overwintering of subterranean parts of herbaceous plants. The recent papers by Nelson (*15*), Leukel (*12*), Albert (*2*), and Graber, Nelson, Leukel, and Albert (*6*) dealing with the relation of root reserves to winterkilling of alfalfa are of interest here. Publications that have a special bearing on some particular phase of the work described in this paper will be mentioned in the discussion.

METHODS

Although most of the alfalfa plants employed in these experiments were of the Kansas Common variety, several other varieties and strains were also used. A strain of Kansas Common certified seed grown near Manhattan, Kans., was used throughout these experiments. The seed of the other varieties and strains was supplied by H. L. Westover, of the United States Department of Agriculture. The plants were grown in the field or in the greenhouse at Manhattan, Kans.

---

[1] Received for publication Feb. 2, 1929; issued August, 1929. Contribution No. 283 from the department of botany and plant pathology, Kansas State Agricultural College.

[2] The writer is indebted to S. C. Salmon of the department of agronomy, Kansas State Agricultural College, for the use of his freezing chamber.

[3] Reference is made by number (italic) to "Literature cited," p. 282.

The freezing point of alfalfa root tissue was determined by the thermoelectric method, a 2-junction thermocouple of the needle type having been used. The arrangement of apparatus was similar to that illustrated and described by Harvey (*7*). The readings were made with a Leeds & Northrup portable galvanometer. Sections of alfalfa root tissue about 2 cm. long were used. A hole was made in the tissue by means of a dissecting needle, and the needle of the thermocouple was inserted firmly in the opening. The tissue remained attached to the needle and was suspended in the freezing chamber in that manner. The ether surrounding the freezing chamber was kept at −9° to −10° C.

The killing point of alfalfa root tissue was determined by freezing the roots for different periods of time at different temperatures and under various conditions.

All the potted plants as well as part of those removed from the soil were frozen in the apparatus described by Hill and Salmon (*10*).

The remaining plants were removed from the soil, washed, and placed in a small sheet-iron chamber surrounded with ice and salt. After being frozen the plants were placed in soil in the greenhouse for observation. The potted plants, after being frozen, also were held in the greenhouse, and notes were taken frequently regarding their condition. In all cases unfrozen plants from the same lot were used as controls. Unless otherwise stated, at least 10 plants were included in each test, and several different periods of exposure were tried at each temperature in order to find the time required to kill the plants.

EXPERIMENTAL DATA

FREEZING POINTS OF ALFALFA ROOT TISSUE

Tests were made to determine the variations in the freezing points of alfalfa roots of different diameters, of sections of roots taken at the crown and at 10 cm. below it, and of different parts of the same cross section of a root.

In one experiment portions of root tissue from the crowns of 296 Kansas Common alfalfa plants of various ages (diameters) and collected at different times over a period of six months were frozen. The roots were gathered at about the same hour each time and while held were thoroughly wrapped in newspaper to reduce the loss of water. The thermocouple needle was inserted in the center of the cross section of the root to a depth of 8 to 10 mm. The results are summarized in Table 1.

TABLE 1.—*Freezing points of alfalfa roots determined by the insertion of a thermocouple in the centers of cross sections taken near the crowns*

Roots frozen	Diameter	Average freezing point	Roots frozen	Diameter	Average freezing point
Number	*Millimeters*	*° C*	*Number*	*Millimeters*	*° C*
1	1	−3.00	17	8	−2.14
13	2	−2.78	17	9	−2.09
31	3	−2.48	23	10	−2.26
42	4	−2.42	13	11	−2.12
57	5	−2.12			
43	6	−2.09	296	-------	*−2.26
39	7	−2.09			

* Average.

These data show a low but statistically significant correlation ($r = 0.215 \pm 0.038$) between the diameters of the roots at the crowns and the depressions of the freezing points. Regression, however, is probably nonlinear, as shown by an inspection of Table 1 and also as indicated by the comparative values of the correlation coefficient (r) and the correlation ratio (η). ($\eta = 0.3568 \pm 0.051$ and 0.5131 ± 0.043; and $\zeta = 0.2171 \pm 0.1504$ and 0.8265 ± 0.3000.) The low correlation might have been due to the fact that the plants were of different ages and were growing under a variety of conditions with respect to moisture and temperature. To test this hypothesis, 42 plants of the same age, growing under the same conditions, were frozen. Two sections from each root were used, one from the crown and the other from 10 cm. below the crown. The diameters of these roots at the crown varied from 2 to 11 mm., and their freezing points ranged from $-1.5°$ to $-3.2°$ C. The correlation coefficient (r) for the sections taken at the crown was 0.094 ± 0.103, and that for those 10 cm. lower was 0.037 ± 0.104. This extremely low correlation may have been due, at least in part, to the small number of samples and to the fact that these random samples did not form a normal distribution curve. Most of the sections from the crown were 5 and 6 mm. in diameter, while a large part of the lower ones were 3 and 4 mm. thick. The remainder were distributed sparingly over a comparatively wide range. Eta was not calculated in these cases because of the small number of variables.

Another phase of this work was a comparison between the freezing points of the roots at the crowns and at 10 cm. below them. The 42 plants under consideration froze at average temperatures of $-2.35°$ C. and $-2.49°$ C. at the crowns and at 10 cm. below, respectively. The probable errors of these two samples were ± 0.025 and ± 0.035, in the order mentioned. The difference between the averages of the freezing points was approximately 3.2 times the difference of their probable errors.

In order to obtain further evidence regarding the relation between the freezing points of the roots near the soil surface and at 10 cm. below, another experiment was conducted with 52 plants. The 52 samples taken at the crown froze at an average temperature of $-2.34°$ C., and a like number from 10 cm. below, at $-2.51°$ C. The freezing points of the sections from the two parts of the same roots varied from 0.1° to 0.3° C. in most cases, although occasionally both sections froze at the same temperature or that from the crown froze at a temperature slightly lower than the one 10 cm. below. The difference between the averages of the freezing points of the tissues of the two sets of samples was approximately four times the difference of their probable errors.

These data seem to show that there is a small but significant difference between the freezing points of the roots at the crown and 10 cm. below it; also that there is a small but statistically significant positive correlation between the diameters and the freezing points of the roots. The general trend of the two sets of data is similar and in the same direction; hence the difference in diameter may account at least in part for the variation in the freezing points of the roots at different levels.

An experiment was made to determine whether the tissue near the cambium froze at the same temperature as that at the center of the root. In this test two thermocouples and two galvanometers were

used. The thermocouple needles were placed in the same piece of root, one in the cambium or in the xylem near it and the other in the center. The tissue froze almost simultaneously throughout, as indicated by the two galvanometer readings. In only 3 out of 24 cases did the tissue of or near the cambium freeze at a lower temperature than that at the center of the root; in 17 it froze at a higher temperature; and in 4 both froze at the same temperature. The average freezing point of the tissue of or near the cambium in 24 roots was −2.11° C., and that at the center of the root was −2.30° C. It should be stated that 20 of the roots used were 3 years old and the other 4 were 4 months old. However, approximately the same relative difference between the freezing points of the tissue at the center and that of or near the cambium was maintained in both cases.

As the freezing points of the tissues in different parts of the roots were found to vary slightly, in all subsequent experiments, except in cases where the roots were too small, the needle was placed in the xylem near the cambium. In a given experiment an effort was made to have the roots of as nearly the same diameter as possible.

FREEZING POINTS OF ROOTS OF DIFFERENT VARIETIES[4] OF ALFALFA

Several experiments were conducted in which sections taken from the taproots near the crowns of different alfalfa varieties were frozen as in previous experiments. It was thought that perhaps some correlation would be found between the freezing points of the tissues and the observed hardiness of the varieties.

These experiments are summarized in Table 2. The figures represent the average of those obtained by freezing 5 to 10 plants. Young roots from the fields were frozen on November 12, when they were in a nonhardened condition, and again on February 11, when they were supposedly more hardened. The soil was frozen on February 11 and had been frozen for at least three days prior to that date. The air temperature for the same period varied from 30° to 4° F. (−1.1° to −15.6° C.) and averaged about 20° F. (−6.7° C.). Although the surface of the soil had been frozen slightly at times previous to November 12, it had usually thawed during the day, and there was no frost in the soil when the plants were dug. The air temperature, although cool at night, had been above freezing for several days except on the nights of November 8, 9, and 10, when it reached a minimum of 23° F. (−5° C.), 22° F. (−5.6° C.), and 26° F. (−3.3° C.), respectively. On the night of November 11 the minimum air temperature was 39° F. (3.9° C.).

The data given in Table 2 show that on November 12 the supposedly nonhardened plants, with the exception of the Hairy Peruvian, froze at lower temperatures than those frozen on February 11. The differences in several cases, however, are small and well within the limits of experimental error. These figures fail to show a correlation between the freezing point and the degree of hardening of the tissues. Furthermore, little or no correlation is shown between the freezing point of the tissue and the known winter hardiness of the varieties. Of those plants frozen on February 11, the Hairy Peruvian, which is perhaps the least hardy variety included, froze at a temperature lower than any of the others except Kansas Common; whereas Cossack, a hardy variety, froze at a higher temperature than any except Utah. There does seem to be some correlation between the

[4] "Variety" as used in this paper includes regional strains.

freezing points and the known hardiness of the varieties frozen on November 12; for example, Cossack, Kansas Common, and Grimm, which are all fairly hardy under Kansas conditions, froze at the lowest temperature, while the two Peruvian varieties, which are the least hardy, froze at the highest temperature. It is thought that this correlation may have been more or less accidental, as it did not hold consistently in other trials.

The average freezing points of 5-year-old roots from the same plots growing on the agronomy farm at Manhattan, Kans., on four different dates are also listed in Table 2. The figures for September 13–15 show a condition that occurred frequently, namely, that when roots were taken from extremely wet soil their freezing point was high. Under such conditions there was little undercooling and the exact freezing point was often difficult to ascertain. Many of these 5-year-old roots had been badly frozen during previous winters and were decaying, so that sometimes it was difficult to get consistent readings. This was especially true on February 3, when the work was discontinued although all the varieties were not then frozen. In these tests the thermocouple needle was always placed near the cambium. The figures in Table 2 show that on September 13–15, Kansas Common froze at a lower temperature than any other variety, while Italian, a nonhardy variety, ranked second; and, with the exception of Cape Lucerne and Argentine, Cossack, a hardy variety, froze at the highest temperature. Of the roots frozen September 19–21, 1926, Grimm, one of the hardiest varieties, froze at the highest temperature. Likewise, of the varieties frozen September 26–28, with the exception of Spanish, Grimm froze most easily. However, Grimm ranks as the hardiest among those varieties frozen on February 3, when the plants should have been in a hardened condition. Obviously, these data fail to show any consistent correlation between hardiness and the freezing point of the root tissue. This confirms the conclusions of Steinmetz (*19*), working with alfalfa, as well as those of other investigators working with other plants.

Many interesting data have been published by Wright and Harvey (*24*), Wright and Taylor (*25*, *26*), Harvey and Wright (*8*), Diehl and Wright (*5*), Wright (*22*), Wright and Diehl (*23*), and others, showing that the freezing and killing points of various fruits and vegetables are practically the same.

The absence of any such correlation between hardiness and the freezing point of the alfalfa roots was further shown by an experiment conducted on February 10, 1927, in which only plants of the Kansas Common variety were used. There were four lots of plants, as follows: (1) 10-months-old plants brought from the field into the greenhouse on December 1, 1926; (2) plants of the same lot obtained from the field on February 10, 1927; (3) 5-months-old plants growing in large pots in the greenhouse; and (4) 5-months-old plants growing in large pots outside the greenhouse. The plants in lots 1 and 3 and in lots 2 and 4 should have been in a nonhardened and a hardened condition, respectively. The average freezing points of 10 plants of each lot were −3.36°, −3.1°, −2.59°, and −2.65 C. for lots 1, 2, 3, and 4, respectively. In this test the nonhardened plants in lot 1 froze at a lower temperature than the hardened ones in lot 2, whereas the nonhardened plants in lot 3 froze at a higher temperature than the hardened ones in lot 4.

TABLE 2.—*Average freezing points (° C.) of the root tissues of different varieties of alfalfa*

Date of freezing	Age of plants	Freezing points of varieties indicated															Condition of soil
		Cossack	Argentine	Hairy Peruvian	Smooth Peruvian	Kansas Common	South African	Utah	Grimm	Ladak	Spanish	Provence	Turkestan	Dakota 12	Cape Lucerne	Italian	
Nov. 12, 1926	3 months	−2.6	−2.35	−2.1	−2.25	−2.65	−2.4	−2.5	−2.85								Wet.
Feb. 11, 1927	6 months	−2.1		−2.5	−2.2	−2.6	−2.3	−2.1									Do.
Sept. 13–15, 1926	5 years	−1.5	−1.5			−2.37		−1.83	−1.72	−1.75	−1.65	−2.17	−1.6	−2.1	−1.0	−2.2	Very wet.
Sept. 19–21, 1926	do	−2.35	−2.2			−2.15		−2.62	−1.76	−2.96	−1.77	−2.0	−2.15	−2.15	−2.55	−2.5	Medium wet.
Sept. 26–28, 1926	do	−2.75	−2.78			−2.73		−2.81	−2.37	−2.71	−2.09	−3.1	−3.01	−2.72	−2.71	−2.77	Medium dry.
Feb. 3, 1927	do	−2.6				−2.17			−2.7	−2.5	−2.38						Wet.

THE KILLING POINTS OF ALFALFA ROOT TISSUE

The period of exposure which plants from the field will withstand varies considerably with their hardiness and with the conditions of the environment. For many years efforts have been made by investigators to develop a method that will enable them to acquire information regarding the conditions that influence the hardiness of plants and the effect of environment on the power of plants to withstand freezing. Most workers seem ultimately to reach the conclusion that some method of artificial freezing is the most satisfactory way to make such studies. The writer has frozen many alfalfa plants in order to learn something of the period of exposure necessary to kill them under different conditions.

Plants Growing in Pots

In these experiments some of the plants used were grown in pots in the greenhouse and the others were obtained from the field. The plants frozen on December 20, 1926, in the last experiment recorded in Table 3 had not grown in pots, but were brought from the field and set in soil in pots just before they were placed in the freezing chamber. In the next to the last experiment listed in Table 3, the plants were growing in 12-inch pots and, because of the insulating effect of the soil, were able to withstand a much longer exposure to a comparatively low temperature.

Table 3.—*Temperatures and exposures necessary to kill Kansas Common alfalfa plants of different ages*

[Plants exposed while growing in 8-inch pots except for those frozen December 12, 1926, which were growing in 12-inch pots]

Date of freezing	Source of plants	Age of plants	Probable state of hardening	Temperature		Period of exposure required to kill plants
				Average	Range	
		Months		°C.	°C	*Hours*
Feb. 16, 1927	Grown in field 7 months, and brought to greenhouse Dec. 1, 1926.	9	Nonhardened.	−20.5	−18 to −22	4½
Dec. 22, 1926	....do....	7½	....do....	−23	−21 to −26	4 to 5
Jan. 25, 1927	....do....	8	....do....	−9.2	−5 to −18	7½
Feb. 28, 1927	....do....	9	....do....	−6.5	−5 to −8	Undetermined.*
Dec. 10, 1926	Always grown in greenhouse....	14	....do....	−26.5	−23 to −30	3½
Dec. 20, 1926	....do....	14	....do....	−23	−21 to −26	3½
Jan. 26, 1927	....do....	15	....do....	−18.5	−5 to −21.4	¼4
Jan. 25, 1927	....do....	15	....do....	−9.2	−5 to −18	7½
Dec. 12, 1926	....do....	8	....do....	−20.5	−18 to −22	24
Dec. 20, 1926	From field at time of freezing....	8	Hardened....	−23.5	−21 to −26	6

* Little injury in 8 hours.

The data presented in Table 3 show that under the conditions of these experiments nonhardened alfalfa roots growing in 8-inch pots were killed in from 3½ to 7½ hours at average temperatures varying from −26.5° to −9.2° C., respectively. Hardened plants from the field and nonhardened plants from the greenhouse, both frozen on December 20, 1926, withstood approximately the same average temperatures, namely, −23.5° and −23° C., for 6 and 3½ hours, respectively. The plants from the field showed considerably more resistance to cold than did those from the greenhouse; this can be explained

only by a variation in the degree of hardening. The nonhardened plants in 12-inch pots required freezing for 24 hours at an average temperature of −20.5° C. to kill them. Here the difference must be attributed to the larger bulk of soil about the roots.

Plants Removed from the Soil

Concomitant with the investigations discussed above, tests were made to determine the killing point of alfalfa roots when removed from the soil. Plants from the greenhouse and from the field were used. The former were nonhardened and the latter varied in degree of hardening. The degree of hardening of the field plants was estimated by studying the thermograph records of the temperatures covering a period of a few days prior to the time the plants were collected. When the temperature ranged from 0° to −20° C. for several days before the plants were frozen they were classed as hardened; when the temperature was above 0° C. for several days they were considered nonhardened. Slightly hardened plants were those collected after a cold period followed by warm weather for a day or two immediately preceding the day the plants were frozen.

The data presented in Table 4 show that the time necessary to kill small plants removed from the soil and frozen varies with the temperature, with the degree of hardening, and to some extent with the size of the roots. The plants grown in 12-inch pots out of doors until autumn and then in the greenhouse were killed in from 15 minutes to 3½ hours at average temperatures of −5.5° to −20° C., depending upon the temperature. The plants which had grown in the field for seven months and in the greenhouse from December 1, 1926, to February 16, 1927, withstood an average temperature of −20° C. for 30 minutes, while the plants frozen on the same day and at the same temperature, recorded as the first line of Table 4, were killed in 15 minutes. The only difference that could be detected between these two lots of plants was in the diameters of their roots. The roots of the plants that withstood exposure for 30 minutes were twice as thick as those that were killed in 15 minutes. This difference was due to the fact that the former had grown in rows in the field for seven months, while the latter, although of about the same age, had grown in pots.

Plants taken from the field and considered to be hardened were frozen on December 22, 1926. These withstood an average temperature of −23.5° C. for three and one-half hours, which was a considerably longer time at a lower temperature than that endured by those of the same lot frozen on March 18 or February 16, 1927. March 18 and February 16 were preceded by two weeks and two days of warm weather, respectively, while December 22 followed a rather protracted cold period.

The effect of hardening is shown also in the results of freezing plants from seed sown broadcast in the autumn. These plants were fairly small, the taproots near the crowns measuring from 2 to 5 mm. in diameter. Two lots of these plants frozen on March 18 at temperatures of −17° and −18.5° C. were killed in 15 minutes. Another slightly hardened lot frozen on February 28 withstood an average temperature of −5° C. for five and one-fourth hours. On the other hand, the slightly hardened plants frozen on February 16 were killed in 30 minutes at −21° C.

TABLE 4.—*Temperatures and lengths of exposure necessary to kill young Kansas Common alfalfa plants of different degrees of hardening*

[Plants removed from the soil and placed in the freezing chamber at the recorded temperatures]

Date of freezing	Source of plants	Age of plants	Probable degree of hardening	Temperature		Period of exposure required to kill plants
				Average	Range	
		Months		°C.	°C	
Feb. 16, 1927	Grown in 12-inch pots out of doors until autumn, then moved into greenhouse.	8	Nonhardened.	−20	−19 to −21	15 minutes.
Mar. 21, 1927	do	9	do	−18.6	−18.2 to −19	25 minutes.
Feb. 25, 1927	do	8	do	−15.6	−15.2 to −16	50 minutes.
Feb. 28, 1927	do	8	do	−5.5	−5 to −6	3½ hours.
Feb. 16, 1927	Grown in field seven months; transferred to greenhouse Dec. 1, 1926.	9	do	−20	−19 to −21	30 minutes.
Mar. 18, 1927	Grown from spring seeding; sown in rows in field.	11	do	−17		50 minutes.
Feb. 16, 1927	do	10	Slightly hardened.	−20.2	−19 to −21.5	1¼ hours.
Dec. 22, 1926	do	8	Hardened	−23.5	−21 to −26	3½ hours.
Mar. 18, 1927	Grown from seed sown broadcast in autumn in field.	7	Nonhardened.	−17		15 minutes.
Do	do	7	do	−18.5		Do.
Feb. 16, 1927	do	6	Slightly hardened.	−21		30 minutes.
Feb. 28, 1927	do	6	do	−5	−4 to −8	5¼ hours.

The data presented in Tables 3 and 4 show that plants from the field varied in hardiness during the winter months. This variation in the time the plants endured a given temperature without being killed is correlated with the temperature to which they were exposed before they were frozen. Plants collected during a cold period were not so easily killed as those collected after a few warm days.

These data also show that the insulating power of the soil is an important factor in protecting the roots from freezing, since nonhardened plants removed from the soil before being frozen often were killed in 15 minutes, whereas they withstood the same temperature for three and one-half hours or longer when growing in soil in 8-inch pots.

A few tests were made during the winter of 1927–28 to determine the comparative hardiness of field and greenhouse plants. The plants were removed from the soil, washed, fixed between 12-inch pot labels in such a way that every other plant belonged to each lot being tested, and then placed top down in the freezing chamber cooled with ice and salt. The plants used were of the same age, but those from the greenhouse were larger, since they had continued to grow after those from the field had ceased to grow because of the low temperature. The field plants used in the experiments conducted on November 11 and January 10 were found to be no hardier than those from the greenhouse. However, on January 17 and 25 the former were less severely injured at a given temperature than the latter. On February 9 the difference in hardiness between the two sets of plants was very slight.

Less striking differences were obtained between the hardened and nonhardened plants in these experiments than in those recorded in Table 4. The differences probably would have been more pronounced, however, if the greenhouse plants had not been larger than those from the field.

FACTORS AFFECTING THE FREEZING OF ALFALFA ROOTS

Diameter of Root

Certain factors other than the hardiness of the plants must be taken into account in studying the freezing of alfalfa roots. For example, other things being equal, large roots usually require a longer time to freeze, as indicated by thermocouple tests, and also withstand a longer exposure at a given temperature without being killed, than do smaller roots. When not properly insulated, the small ends of the taproots of both hardened and nonhardened plants are killed first when subjected to experimental freezing. This seldom happens in the field, for there the cold can reach the roots only from above. Figure 1 shows two plants with the lower ends of the taproots killed by freezing. These plants grew in a 12-inch pot placed outside the greenhouse during the winter. A number of plants near the margin where they were not so well protected with soil showed this condition. Roots of alfalfa plants removed from the soil and then plunged into a freezing chamber often died from below upwards in this manner, so that plants whose crowns were apparently uninjured eventually died. There is another possible explanation of the freezing of alfalfa roots from below under certain conditions, namely, that the lower part of the root which is not subjected to such low temperatures during the hardening period is not so hardy as the upper part. In order to avoid this type of injury a freezing chamber which was open at the top was devised, and the plants were placed in it with the tops down. In this way the tops and upper parts of the taproots were exposed to the lowest temperature. This type of freezing chamber was used almost exclusively during 1927–28.

Rate of Thawing

Still another factor which is thought by many to influence the killing of plants by freezing is the rate of thawing. No detailed study of this factor was made, but it was tested to some extent with respect to alfalfa by freezing young plants of the Kansas Common and Grimm varieties growing in pots at different temperatures and for different lengths of time. About 1,600 plants in all were used. One half of these were frozen and then thawed quickly in a warm greenhouse, while the other half were wrapped in felt, covered with sawdust, and kept in a cool basement for 24 hours or longer. Subsequently both lots were treated alike. No difference in the amount of killing was evident in any case. This does not prove that the rate of thawing is never a factor in winterkilling, but it does indicate that rapid thawing does not necessarily cause a greater amount of injury.

Root Reserves

A correlation between the amount of root reserves and the longevity of alfalfa stands has been observed by a number of agronomists. This has received special attention in recent years in Wisconsin (*2*, *6*, *12*, *15*) and in Minnesota (*19*). However, these workers do not seem actually to have demonstrated that alfalfa plants with low carbohydrate root reserves freeze more easily than similar plants with an abundance of such reserves. In order to obtain some definite information on this point the following experiment was conducted: Nine 8-inch pots in which were growing about ten 5-months-old

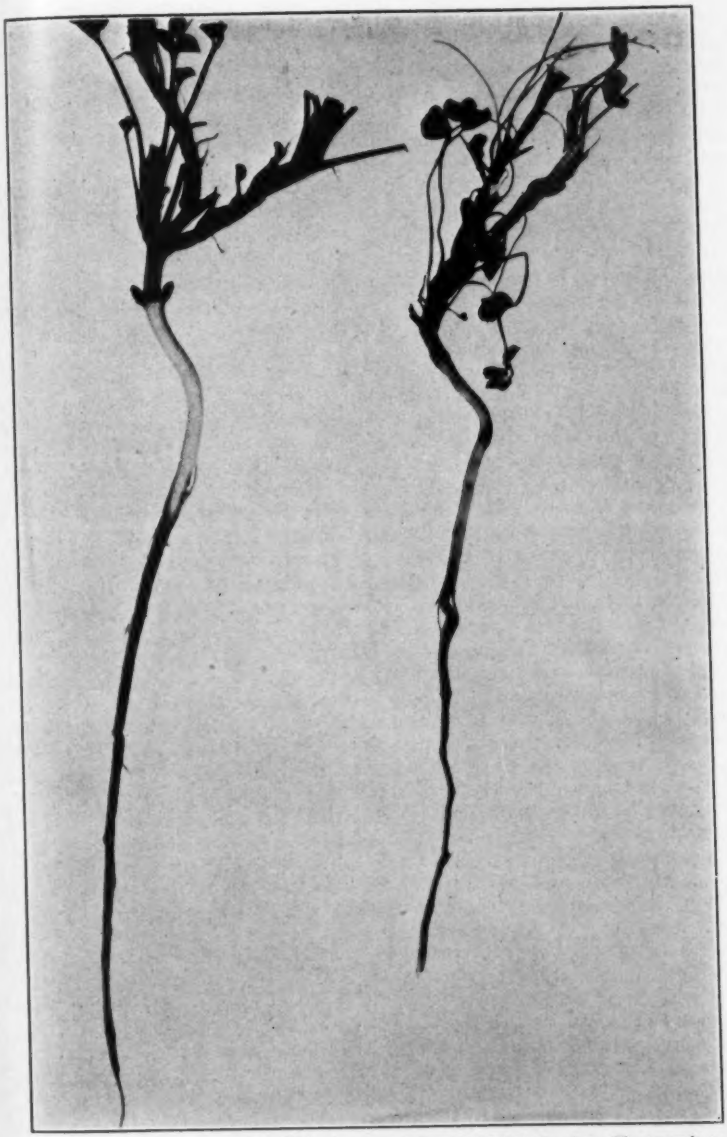

FIGURE 1.—Two alfalfa plants with the lower parts of their roots killed by freezing. They grew in a 12-inch pot outside the greenhouse during the winter. A number of plants near the margins of the pots where they were not well protected by soil were affected in this manner

Kansas Common alfalfa plants were held in the greenhouse in a box covered with heavy black threaded felt. This was shaded with newspaper to prevent the temperature from becoming too high. The plants were thus deprived of light but were provided with moisture and a temperature somewhat comparable, although probably slightly higher at times, than that to which the control plants were subjected. The control consisted of an equal number of pots from the same lot held near the box but left uncovered. The plants were placed in the box on January 24, 1928.

On January 31, when the first pots were removed from the box, the leaves had lost perhaps 75 per cent of their chlorophyll. The plants were removed from the pots, the tops cut off about 2 inches from the crowns, and the roots trimmed so as to leave taproots about 6 inches in length. These plants, together with those from the control pots treated in a similar manner, were placed tops down in the freezing chamber. Three lots of five plants each were frozen for 10, 15, and 20 minutes at temperatures of $-5°$ to $-6°$ C., $-6°$ to $-8°$ C., and $-9°$ to $-10°$ C., respectively, after which they were planted in a bed in the greenhouse. The plants frozen for 10 and 15 minutes were injured, but little or no difference was evident between those that had been deprived of light and the controls. However, plants frozen 20 minutes showed injury estimated at 75 per cent, as compared with 66 per cent for the controls.

In like manner plants from both sets of pots were frozen on February 9, and those that had been held in the dark were more severely injured than the controls. On this date the original top growth was about dead and new white shoots were being produced.

A third freezing was made on February 17, at which time the original tops of the plants held in the dark were dead and the new white shoots were 6 inches long. Plants of both lots were frozen for 15 minutes at temperatures ranging from $-4.5°$ to $-9°$ C. The control plants all lived, although two were weakened considerably, while all but two of the plants from the dark died. All plants of each lot treated in exactly the same way as the others except that they were not frozen grew well. A photograph of a pot from the box and a control pot taken March 3 is shown in Figure 2, A. The remaining plants were frozen on March 4, and those which had been deprived of light were much more severely injured than the controls.

This single experiment, although conducted on a very limited scale and with nonhardened plants, indicates that plants lacking root reserves are killed more easily by freezing than those with abundant root reserves. Judging from the data presented by other investigators (*2*, *6*), the carbohydrate root reserves in the plants held in the dark in the writer's experiment probably were not entirely depleted even at the end of the experiment. However, a study of cross sections of some of the roots showed that the starch was entirely gone in plants deprived of light (fig. 2, C) and that it was very abundant in the control plants (fig. 2, B). No analyses were made to determine whether or not sugar was present in the former lot of plants. Since roots whose carbohydrate reserves were reduced by being deprived of light froze more easily than the controls, it seems probable that plants whose root reserves have been decreased by too frequent cuttings will likewise freeze more easily than plants with abundant root reserves.

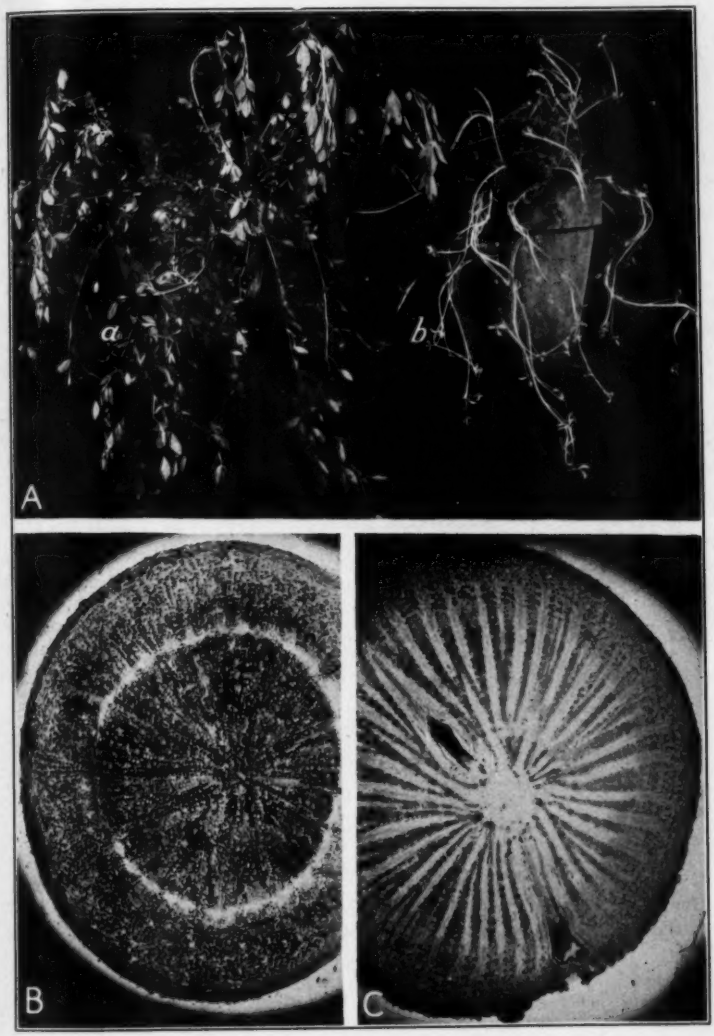

FIGURE 2.—A, Two pots of Kansas Common alfalfa plants of the same age. One pot (*a*) had grown continuously in the greenhouse, while the other (*b*) had been deprived of light by being inclosed in a box covered with black paper. The original tops of the plants in *b* died, and new white shoots were sent out. Such plants when transplanted to a bed in the greenhouse grew practically as well as those of the controls. B, Cross section of one of the roots in the control pot in A, *a*, showing the cells filled with starch as denoted by the black color. The starch was stained with iodine before being photographed. × about 8. C, Cross section of one of the plants held in the dark box, showing the complete absence of starch. Plants deprived of light were more easily killed by freezing than were plants grown in the light. × about 8.

Environmental Conditions

Soil Moisture

The moisture content of the soil markedly influences the resistance of potted plants to freezing injury. Hill and Salmon (*10*) found that when wheat plants growing in dry soil were frozen under artificial conditions, they were injured much more severely than similar plants growing in wet soil. This difference they attributed to the high specific heat of water, which in a wet soil prevents a rapid change in temperature with the result that plants in dry soil are exposed to a lower temperature than those in wet soil.

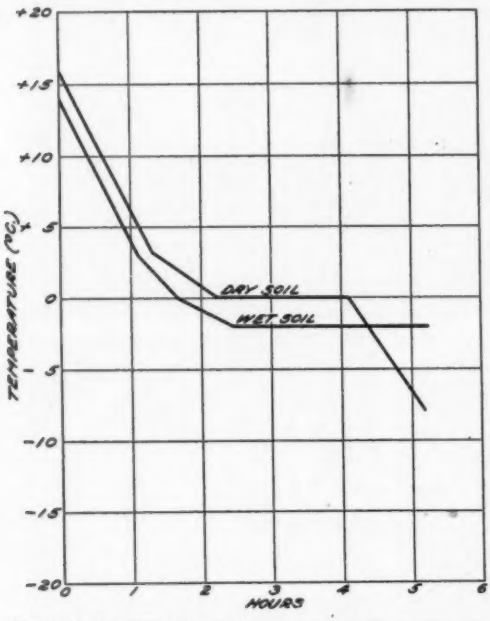

FIGURE 3.—Comparative rate of temperature fall in wet and dry soil contained in 8-inch pots and placed in a freezing chamber which had an original temperature near 0° C., but which was lowered rapidly to —10° C. and more slowly to — 16°C.

The extent to which the moisture may influence the lowering of the temperature of the soil was illustrated in an experiment conducted by the writer in which two 8-inch pots, one with wet and the other with dry soil, were placed near each other in a freezing chamber. A thermometer was placed in the soil in the center of each pot, and frequent readings were made. The temperature of the wet soil dropped to 0° C. in 1⅔ hours. In another 50 minutes it reached −2° C., and it remained at that temperature throughout the experiment, or for about 2⅔ hours longer. On the other hand, the dry soil required 2¼ hours to reach 0° C., where it remained for 1⅝ hours, and then dropped to −8° C. in 65 minutes. Hence the temperature in the wet soil reached −2° C. while that in the dry soil reached −8° C. in approximately 5⅛ hours. The complete data are presented graphically in Figure 3. These results simply show what happened in one instance; the effect of moisture would of course vary with the amount of water in the soil and perhaps with other factors.

Four 8-inch pots with plants growing in them were placed in the freezing chamber with those described above. The soil in two of the pots was wet and that in the other two dry. Two pots, one with wet and one with dry soil, were removed after 4½ and 5⅛ hours, respectively. The plants in the wet soil in the pot removed after 4½ hours were uninjured, while those in the dry soil were all dead. The plants

in the dry soil in the pot removed after 5¼ hours were also dead, while only 3 of the 10 plants in the wet soil succumbed. The other 7 plants in the wet soil withstood an exposure of 5¼ hours to a temperature that killed all the plants in the dry soil in less than 4½ hours. The temperature of the air in the freezing chamber during this test ranged from +1° to −16° C. It reached −9° C. in the first half hour and then went down gradually throughout the experiment. This experiment shows how great an error may be made in interpreting the results of freezing plants under experimental conditions if the soil moisture is not taken strictly into account. No doubt soil texture with its varying water-holding capacity may also influence the results of experimental freezing tests.

AIR AND SOIL TEMPERATURES

Steinmetz (*19*) states that in Minnesota plants brought from the field in January gave no marked evidence of having been killed during the winter. According to his observations, dead plants first appear at the time of the spring thaw in March. He states that "it may well be questioned whether repeated freezing and thawing occurs under natural conditions in the soil." No doubt he refers to Minnesota conditions, for the soil may freeze and thaw several times during a winter in Kansas. Plants killed by freezing have been seen in Kansas in January and February, and plants badly injured have been found in December. On February 24, 1927, the roots of some nonhardy strains of alfalfa were observed to be entirely softened and disintegrating, having, no doubt, been dead for some time.

Different varieties of alfalfa, including Kansas Common and Grimm, growing in the field showed considerable injury on December 12, 1927, following a drop in the air temperature, as recorded by the thermograph, to −9° F. (−22.8° C.) and the temperature of the soil 2 inches below the surface of 24° F. (−4.4° C.). Frequently serious injury to alfalfa plants is overlooked for a time unless a microscopic study of sections of the root is made. Usually alfalfa in Kansas is not protected by snow during cold periods, as it is in some more Northern States. Although some heaving of the roots occurs, the most serious effects of freezing in this State and in some other States in which observations have been made seem to be the injury produced in the crown and in the upper part of the taproot. The condition of roots badly injured by freezing has been described in detail by the writer (*20*) [5] and by Jones (*11*). These injuries often so greatly reduce the amount of active tissue that the plant makes little growth and eventually succumbs. These injuries also appear to provide avenues through which microorganisms, especially *Aplanobacter insidiosum* L. McC., may enter and hasten the death of the plant.

As has been stated, the injury to alfalfa plants from freezing is largely limited to the upper part of the taproot and crown. This is due to the fact that the part of the root lower down is protected by the soil. In order to learn more about the rate of temperature fall of the root as compared with that of the air, the following experiment was conducted. A pot in which young alfalfa plants were growing was placed in the freezing chamber. A thermometer was placed in the soil near one of the plants just deep enough to cover the bulb,

[5] WEIMER, J. L. INJURIES OF ALFALFA ROOTS RESULTING FROM FREEZING. [Unpublished manuscript.]

and a thermocouple needle was inserted into the crown of the plant about 1 cm. above the surface of the soil. A record of the temperature falls in the soil, in the air, and in the plant was kept. These are shown graphically in Figure 4. These data show that the soil temperature lagged behind the air temperature several degrees until the former reached 0° C., after which it cooled more rapidly, being only 8 degrees higher than the air temperature at the termination of the experiment. The temperature of the plant took up an intermediate position between that of the air and of the soil. This experiment indicates that the temperature of the alfalfa roots at or slightly above the surface of the soil may drop much more slowly than the air temperature and under some conditions may not reach a temperature as low as that of the air for a long time.

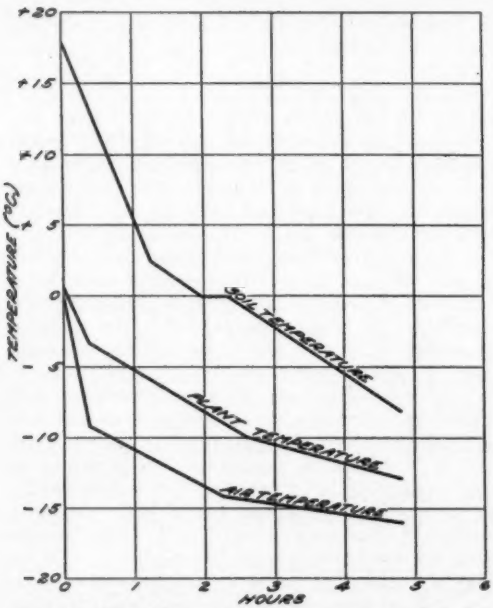

FIGURE 4.—Comparative rate of temperature fall of the soil in an 8-inch pot, of the plant growing in the pot (the record being taken 1 cm. above the soil), and of the surrounding air. The temperatures of the plant and of the soil were originally the same, but by the time the first record was made the plant had cooled down to near the air temperature

Further information regarding the reason why alfalfa roots freeze only near the surface of the soil is obtained by an examination of thermograph records kept at Manhattan, Kans., since 1926. The soil temperature was taken at a depth of 2 inches. The minimum air and soil temperatures for the coldest periods of the winters 1926–27 and 1927–28 are recorded in Table 5. There were two cold periods in the winter of 1926–27, one in December and the other in January. The air temperature in December, 1926, did not go much below 0° F. (−17.8° C.), but the soil temperatures on December 15 and 16 were comparatively low. The air temperature dropped to −14.5° F. (−25.8° C.) on January 15, 1927, but the soil temperature went down only to 25.5° F. (−3.6° C.) owing to a thin covering of snow. There were three comparatively cold periods during the winter of 1927–28, two in December and one beginning on December 31 and extending into January. The first decided drop in temperature came on December 7 and 8, when the air temperatures reached 0° and −9° F. (−17.8° and −22.8° C.), respectively. As this cold period was of rather short duration, the soil temperatures did not go very low. However, a histological study of plants from the field showed that

even in the more hardy varieties some splitting along the rays had taken place, while the nonhardy varieties were badly injured. The next cold period, which extended from December 16 to 23, was the most severe, and it seems probable that much of the winterkilling of that season occurred during this time. The minimum air temperature did not go below 0° F. (−17.8° C.) except on two nights during this period, although it approached 0° F. on six of the eight nights. The soil temperature went lower at this time than at any other time during the two years. This may have been due to one of two factors, or to a combination of the two, namely, the duration of the cold period or the dryness of the soil. Only 0.09 inch of rain fell in November and 0.57 inch of rain and 0.07 inch of snow fell in December, 1927, according to the weather records for Manhattan, Kans. It should be noted also that the air temperatures did not go so low as in January, 1927.

TABLE 5.—*Minimum air and soil temperatures at Manhattan, Kans., during the coldest periods of the winters of 1926–27 and 1927–28*

Date	Minimum air temperature	Minimum soil temperature 2 inches below surface	Remarks
	°F.	°F.	
Dec. 13, 1926	+4	+25	No snow.
Dec. 14, 1926	0	+20	Do.
Dec. 15, 1926	−2	+17	Do.
Dec. 16, 1926	+7	+18	Do.
Jan. 14, 1927	0	+26	Some snow on ground.
Jan. 15, 1927	−14.5	+25.5	Do.
Dec. 7, 1927	0	+24	No snow; soil very dry.
Dec. 8, 1927	−9	+24	Do.
Dec. 16, 1927	+2	+20	Do.
Dec. 17, 1927	+12	+20	Do.
Dec. 18, 1927	0	+17	Do.
Dec. 19, 1927	−2	+16	Do.
Dec. 20, 1927	−2	+14	Do.
Dec. 21, 1927	+1	+15	Do.
Dec. 22, 1927	+14	+20	Do.
Dec. 23, 1927	+1	+15	Do.
Dec. 31, 1927	−10	+22	Thin layer of snow on ground.
Jan. 1, 1928	−12	+19	Do.
Jan. 2, 1928	−8	+20	Do.
Jan. 3, 1928	−16	+18	Do.
Jan. 4, 1928	−2.5	+19	Do.
Jan. 5, 1928	+8	+20	Do.

The final cold period for the winter of 1927–28 began December 31, 1927, and lasted until January 5, 1928. The air temperatures during this period were the lowest experienced during the two years, but the plants were protected somewhat by a thin layer of snow, which kept the soil temperature from going as low as it had done previously.

The data in Table 5 show that the temperature of the soil at a depth of 2 inches remained very much higher than that of the air during the coldest periods experienced at Manhattan in the two winters during which records have been kept. An examination of alfalfa plants of different varieties showed that on January 14 India was entirely killed and several other varieties such as Arizona, South African, Spanish, Smooth Peruvian, and Hairy Peruvian were severely injured. More or less splitting of the rays or other parts of the roots was seen in most of the varieties studied. When growth began in the spring, however, only those varieties listed above showed much winterkilling. It should be added that there was only a moderate

amount of winterkilling at Manhattan during the winter of 1926–27 and also during the following winter.

Bound Water as a Measure of Cold Resistance

Rosa (*18*) and Lott (*13*) have used the dilatometer method for determining the amount of nonfreezable water in plant tissue. Both report having found a correlation between the amount of nonfreezable or bound water and the hardiness of the tissues with which they worked. Steinmetz (*19*), using a method described by Newton and Gortner (*17*), found no correlation between the bound water and winter hardiness in alfalfa plants.

Following the method described by Rosa and Lott, the writer tested a number of alfalfa roots of different varieties in the fall and again in the following February. The plants were all from a plot in which the different varieties were growing in rows. The soil was fairly uniform in texture and in drainage. When an experiment was begun the plants were brought to the laboratory, washed, dried, cut into thin slices, and 10-gm. samples weighed into tared weighing bottles. The tissue was covered with petroleum ether in tightly stoppered bottles and held in the ice box until needed, which was usually not longer than 24 hours. Preliminary tests indicated that the amount of bound water in the samples did not change when held under these conditions for several hours. In most cases all the samples of a series were collected and prepared for use at the same time. However, in one experiment the samples were collected and tested on the same day. A composite sample of 10 to 15 plants was used in each case. The dilatometer was immersed in a salt and ice bath held at about $-5°$ C. Difficulty was experienced in inoculating all the samples at the same temperature. Usually the nonhardy varieties became inoculated at about $-2°$ C. The writer discovered no way which would consistently start the formation of ice crystals in some of the more hardy samples until they had cooled one-half degree or more. This did not always happen, but when it did sometimes a larger amount of water froze than in the nonhardy varieties. At other times, however, only a small amount of water froze, even in samples which did not become inoculated until a temperature of $-3°$ C. was reached.

An examination of Table 6 shows that the hardened plants had a slightly higher water content than the nonhardened ones with the exception of Grimm, Kansas Common, and Spanish. In every case, however, more of the water in the nonhardened plants froze, leaving a smaller amount of unfrozen or bound water. There appears to be, therefore, a correlation between the bound water and the state of hardening of the plants when the two separate groups of plants are considered. However, when the individual varieties within the groups are compared no such correlation can be seen. Of course, no correlation was expected in the nonhardened group. It scarcely seems probable that the plants frozen in February were not hardened, as they were taken from soil frozen 2 inches or more in depth. It must be admitted, however, that little is known as yet regarding the actual time required for the hardening of alfalfa roots. The varieties shown in Table 6 are so arranged that the more hardy appear toward the top and the least hardy lower down. The least

hardy group comprises the five varieties listed last in the table. Hardigan, Cossack, and Grimm are usually considered among the most hardy. The remaining varieties may be considered as intermediate, although some of them are also rather hardy. Of those frozen in February, Grimm, of the hardy group, contained the highest percentage of bound water, while Spanish, Smooth Peruvian, and nonhardy varieties ranked next. On the other hand, Cossack, a hardy variety, had the least bound water. Hardiness in these experiments is not necessarily correlated with low moisture content, for, as pointed out, the plants of the hardened series contained slightly more water than the others. On the other hand, Lott (*13*) found that raspberry plants having a lower moisture content also had a higher percentage of bound water. Rosa (*18*) likewise obtained an increase in bound water with a decrease in the water content of the tissue.

TABLE 6.—*Quantity and state of the water in 10-gram samples of different varieties of alfalfa, when hardened and nonhardened*

[Hardened plants frozen February, 1928; nonhardened ones September, 1927]

Variety	Hardened					Nonhardened				
	Dry weight	Total water	Freezable water	Bound water	Percentage of bound water	Dry weight	Total water	Freezable water	Bound water	Percentage of bound water
	Grams	*Grams*	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*	*Grams*	
Hardigan	3.070	6.930	1.5	5.430	78.354	3.488	6.512	2.9	3.612	55.466
Cossack	2.970	7.030	2.8	4.230	60.170	3.121	6.879	3.6	3.279	47.666
Grimm	2.837	7.163	1.0	6.163	86.039	2.809	7.191	3.4	3.791	52.718
Lebeau	2.788	7.212	1.4	5.812	80.587	2.807	7.193	3.75	3.443	47.865
Turkestan	1.726	8.274	1.5	6.774	81.870					
Kansas Common	2.755	7.245	1.8	5.445	75.155	2.375	7.625	4.0	3.625	47.540
Dakota	2.768	7.232	1.7	5.532	76.493	2.843	7.157	3.8	3.357	46.905
Utah	2.944	7.056	1.4	5.656	80.158	3.022	6.978	3.2	3.778	54.141
Argentine	2.758	7.242	1.6	5.642	77.906	2.795	7.205	4.1	3.105	43.095
Italian	2.799	7.201	2.3	4.901	68.059	2.959	7.041	3.5	3.541	50.291
Spanish	2.622	7.378	1.3	6.078	82.380	2.327	7.673	4.15	3.523	45.914
South African	2.930	7.070	1.7	5.370	75.954	3.503	6.497	3.3	3.197	49.207
Smooth Peruvian	3.003	6.997	1.1	5.897	84.278	3.185	6.815	3.15	3.665	53.778
Hairy Peruvian	2.936	7.064	2.05	5.014	70.979	2.992	7.008	3.1	3.908	55.764

CONCLUSIONS

It may be concluded from the results of these investigations that there is no correlation between the freezing point of alfalfa root tissue when in a hardened condition and its known winter hardiness. The freezing point of roots in a nonhardened condition is often lower than that of hardened roots. Likewise, the freezing point of the root tissue of nonhardy alfalfa varieties is frequently lower than that of hardy varieties under apparently the same conditions.

It is possible to distinguish between plants in a hardened and in a nonhardened condition by freezing the roots of plants growing in pots or by removing the roots from the soil and freezing them.

The amount of bound water in the tissue of hardened and nonhardened alfalfa roots may serve as a measure of their degree of hardening, but it does not necessarily differentiate between hardy and nonhardy varieties.

SUMMARY

The freezing point of alfalfa root tissue was determined by the thermoelectric method. In these tests there was little correlation between the freezing points and the diameters of the roots.

There was a slight correlation between the freezing points of sections of alfalfa taproots taken near the crown and similar sections taken 10 cm. below.

The average freezing point of root tissue in or near the cambium was −2.11° C., while that of the center of the root was −2.3° C.

No correlation was found to exist between the freezing point and the known hardiness of the root tissue of different varieties of alfalfa.

Alfalfa roots under the conditions of these experiments when growing in 8-inch pots were killed in 3½ to 5 hours at temperatures ranging from −20° to −25° C. and withstood average temperatures of −6.5° to −9.2° C. for more than 7 hours. Hardened plants from the field and nonhardened plants from the greenhouse withstood temperatures of −21° to −26° C. for 6 and 3½ hours, respectively.

Many roots were removed from the soil before they were exposed to freezing temperatures. When this was done, small nonhardened roots were killed in 15 minutes at temperatures from −17° to −20° C., while larger roots withstood these temperatures twice as long. The time of exposure necessary to kill roots of small plants varied from 15 minutes to 3½ hours, depending upon the temperature to which they were exposed. The period during which field plants endured temperatures of −17° C. and −23.5° C. (range −21° to −26° C.) varied from 50 minutes to 3½ hours, depending upon their degree of hardening.

The amount of cold which alfalfa roots can withstand depends upon their hardiness, the amount of insulation afforded by the substratum in which they are embedded, soil moisture, and other factors.

Roots of nonhardened plants with abundant carbohydrate reserves freeze less easily than roots of plants that have been deprived of light for some time. Presumably this difference is due to a change in the physiological condition of the cells brought about by a lack of root reserves.

It is shown by using the dilatometer method that a correlation existed between the amount of bound water and cold resistance of hardened and nonhardened plants. A similar correlation with respect to the known hardiness of different varieties, however, was not evident.

LITERATURE CITED

(1) Abbe, C.
1895. The influence of cold on plants—a résumé. U. S. Dept. Agr. Expt. Sta. Rec. 6: 777–781.

(2) Albert, W. B.
1927. Studies on the growth of alfalfa and some perennial grasses. Jour. Amer. Soc. Agron. 19: 624–654, illus.

(3) Blackman, F. F.
1909. Vegetation and frost. New Phytol. 8: 354–363.

(4) Chandler, W. H.
1913. The killing of plant tissue by low temperature. Missouri Agr. Expt. Sta. Research Bul. 8, p. 143–309, illus.

(5) Diehl, H. C., and Wright, R. C.
1925. Freezing injury of apples. Jour. Agr. Research (1924) 29: 99–127, illus.

(6) Graber, L. F., Nelson, N. T., Leukel, W. A., and Albert, W. B.
1927. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wis. Agr. Expt. Sta. Research Bul. 80, 128 p., illus.

(7) HARVEY, R. B.
1919. IMPORTANCE OF EPIDERMAL COVERINGS. Bot. Gaz. 67: 441–444, illus.
(8) ——— and WRIGHT, R. C.
1922. FROST INJURY TO TOMATOES. U. S. Dept. Agr. Bul. 1099, 10 p., illus.
(9) HILDRETH, A. C.
1926. DETERMINATION OF HARDINESS IN APPLE VARIETIES AND THE RELATION OF SOME FACTORS TO COLD RESISTANCE. Minn. Agr. Expt. Sta. Tech. Bul. 42, 37 p., illus.
(10) HILL, D. D., and SALMON, S. C.
1927. THE RESISTANCE OF CERTAIN VARIETIES OF WINTER WHEAT TO ARTIFICIALLY PRODUCED LOW TEMPERATURES. Jour. Agr. Research 35: 933–937.
(11) JONES, F. R.
1928. WINTER INJURY OF ALFALFA. Jour. Agr. Research 37: 189–211, illus.
(12) LEUKEL, W. A.
1927. DEPOSITION AND UTILIZATION OF RESERVE FOODS IN ALFALFA PLANTS. Jour. Amer. Soc. Agron. 19: 596–623.
(13) LOTT, R. V.
1926. CORRELATION OF CHEMICAL COMPOSITION WITH HARDINESS IN BRAMBLES. Missouri Agr. Expt. Sta. Research Bul. 95, 22 p., illus.
(14) MARTIN, J. H.
1927. COMPARATIVE STUDIES OF WINTER HARDINESS IN WHEAT. Jour. Agr. Research 35: 493–535, illus.
(15) NELSON, N. T.
1925. THE EFFECTS OF FREQUENT CUTTING ON THE PRODUCTION, ROOT RESERVES, AND BEHAVIOR OF ALFALFA. Jour. Amer. Soc. Agron. 17: 100–113.
(16) NEWTON, R.
1922. A COMPARATIVE STUDY OF WINTER WHEAT VARIETIES WITH ESPECIAL REFERENCE TO WINTER-KILLING. Jour. Agr. Sci. [England] 12: 1–19.
(17) ——— and GORTNER, R. A.
1922. A METHOD FOR ESTIMATING HYDROPHYLIC COLLOID CONTENT OF EXPRESSED PLANT TISSUE FLUIDS. Bot. Gaz. 74: 442–446, illus.
(18) ROSA, J. T., Jr.
1921. INVESTIGATIONS ON THE HARDENING PROCESS IN VEGETABLE PLANTS. Missouri Agr. Expt. Sta. Research Bul. 48, 97 p., illus.
(19) STEINMETZ, F. H.
1926. WINTER HARDINESS IN ALFALFA VARIETIES. Minn. Agr. Expt. Sta. Tech. Bul. 38, 33 p., illus.
(20) WEIMER, J. L.
1927. OBSERVATIONS ON SOME ALFALFA ROOT TROUBLES. U. S. Dept. Agr. Circ. 425, 10 p., illus.
(21) WIEGAND, K. M.
1906. THE OCCURRENCE OF ICE IN PLANT TISSUE. Plant World 9: 25–39, illus.
(22) WRIGHT, R. C.
1927. SOME EFFECTS OF FREEZING ON ONIONS. U. S. Dept. Agr. Circ. 415, 8 p., illus.
(23) ——— and DIEHL H. C.
1927. FREEZING INJURY TO POTATOES. U. S. Dept. Agr. Tech. Bul. 27, 24 p., illus.
(24) ——— and HARVEY, R. B.
1921. THE FREEZING POINT OF POTATOES AS DETERMINED BY THE THERMOELECTRIC METHOD. U. S. Dept. Agr. Bul. 895, 7 p., illus.
(25) ——— and TAYLOR, G. F.
1921. FREEZING INJURY TO POTATOES WHEN UNDERCOOLED. U. S. Dept. Agr. Bul. 916, 15 p., illus.
(26) ——— and TAYLOR, G. F.
1923. THE FREEZING TEMPERATURES OF SOME FRUITS, VEGETABLES, AND CUT FLOWERS. U. S. Dept. Agr. Bul. 1133, 8 p.

RELATION OF TEMPERATURE OF FERMENTATION TO QUALITY OF SAUERKRAUT [1]

By E. A. MARTEN, W. H. PETERSON and E. B. FRED, *Departments of Agricultural Chemistry and Agricultural Bacteriology, University of Wisconsin*, and W. E. VAUGHN, *Research Department, American Can Co.*[2]

INTRODUCTION

The importance of temperature control in sauerkraut manufacture is becoming better appreciated in factory practice, but until now only cursory observations, rather than accurate measurements under well-regulated conditions, have been made. Since the temperature of cabbage as it comes into the factory may vary from as high as 85° to as low as 32° F., depending upon weather conditions, temperature control has been adopted in many factories. Great differences of opinion exist among sauerkraut packers, however, as to the best temperature at which to carry out the fermentation. Some prefer to operate at a temperature of 70° to 80°, while others let the temperature fall as low as 50°. The higher temperature has the advantage of inducing a rapid formation of acid, but this is offset by a tendency to produce soft and pink sauerkraut. For this reason a temperature must be selected which gives a rapid fermentation without sacrificing the quality of the product.

REVIEW OF LITERATURE

The literature on the relation of temperature to sauerkraut fermentation is both meager and conflicting. Wehmer [3] recommends a temperature of 40° F. Henneberg [4] suggests that 68° to 77° be employed at the start and 50° to 59° during the later stages of the fermentation. Fabian [5] recommends that the fermentation be carried out at 80° in order to give a product of pleasant taste and aroma. LeFevre [6] reports that sauerkraut can be made in from six to eight days by employing a temperature of 80°. The present authors have never been able to make sauerkraut of good quality at this temperature. It was possible to obtain a sufficiently high acid concentration in a short time at this temperature, but the product still possessed the characteristic odor and flavor of raw, sour cabbage rather than that of sauerkraut. At elevated temperatures (75° to 80°) there is also serious danger of producing soft and slimy sauerkraut and at times pink sauerkraut. In a previous study of the relation

[1] Received for publication Mar. 9, 1929, issued August, 1929. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

[2] The authors are greatly indebted to the Frank Pure Food Co., Franksville, Wis., for their cooperation in this work.

[3] WEHMER, C. DIE SAUERKRAUTGÄRUNG. Centbl. Bakt. [etc.] (II)10: [625]-629. 1903.

[4] HENNEBERG, W. DAS SAUERKRAUT (SAUERKOHL). 89 p. illus. Inst. Gärungsgewerbe, Berlin. 1916. [Reprint from Deut. Essigindustrie Jahrg. 20: [133]-136, [141]-144, 152-155, 160-161, 166-170, 176-177, 184-185, 192-194, 199-202, 207-209, 215-216, 223-225, illus. 1916.]

[5] FABIAN, F. W. THE SCIENCE OF MAKING SAUERKRAUT. CARE NEEDED TO EXCLUDE UNDESIRABLE BACTERIA FROM KRAUT—HOME-MADE STARTER MAY BE USED. Mich. Agr. Expt. Sta. Quart. Bul. 9: 50-51. 1926. [Also Market Growers' Jour. 41: 639. 1927.]

[6] LEFEVRE, E. THE COMMERCIAL PRODUCTION OF SAUERKRAUT. U. S. Dept. Agr. Circ. 35, 30 p., illus. 1928.

of temperatures to commercial sauerkraut production, it was reported[7] that the best sauerkraut was produced when temperatures between 55° and 65° existed in the vat. The experiments recorded in this paper are a continuation and extension of the previous work, and the results now presented substantially confirm it as to the best temperatures at which to produce sauerkraut.

EXPERIMENTAL METHODS

LABORATORY EXPERIMENTS

The sauerkraut was made either in 10-gallon stone jars or in 45-gallon barrels. Early Copenhagen and All Seasons cabbage were used. Sixty pounds of cabbage were packed in the jars and 300 pounds in the barrels. Two and one-half per cent of salt was used in all cases. The following three ranges of temperature were studied: −10° to 50°, 55° to 60°, and 70° F.

The fermentations at 70° F. were carried out in a greenhouse where the temperature was maintained by means of thermostat control. The fermentations at 55° to 60° were conducted in a basement, the temperature being regulated by means of steam heat and air circulation. The low-temperature fermentations, −10° to 50°, were made in barrels placed outside of the building where they remained from December to March.

TABLE 1.—*The effect of fermentation temperature on the quality of sauerkraut*

Sample No.	Size of container	Temperature of fermentation	Age of sauerkraut	Final acidity as lactic acid	Quality
		° *F.*	*Days*	*Per cent*	
1	10-gallon jar	60	43	1.98	Good.
2	do	60	43	1.92	Do.
3	do	70	43	2.02	Fair.
4	do	70	43	1.91	Do.
5	45-gallon barrel	−10 to 50	125	.58	Very poor.
6	do	−10 to 50	125	.58	Do.
7	do	55 to 60	35	1.67	Good.
8	do	55 to 60	35	1.72	Do.
9	do	70	35	1.62	Poor.
10	do	70	35	1.62	Do.

Table 1 summarizes the results of the experiments. The sauerkraut made at 70° F. was only fair in quality; that which was made at 55° to 60° was good in every instance, and that which stood outside did not become sauerkraut at all but was merely spoiled cabbage. This material was rotted to the depth of a foot or more at the top and at the bottom, was raw and contained no appreciable amount of acid. Although such extreme conditions do not prevail in the making of sauerkraut the results show in an exaggerated form some of the effects which may be expected when sauerkraut is made at low temperatures.

FACTORY EXPERIMENTS

Other experiments were conducted in a commercial sauerkraut factory. The cabbage used was the All Seasons variety and had been stored outside for about a month in a well-ventilated hay-covered pile

[7] PARMELE, H. B., FRED E. B., PETERSON, W. H., MCCONKIE, J. E., and VAUGHN, W. E. RELATION OF TEMPERATURE TO RATE AND TYPE OF FERMENTATION AND TO QUALITY OF COMMERCIAL SAUERKRAUT. Jour. Agr. Research 35: 1021–1038, illus. 1927.

and had a temperature of about 38° F. when brought to the factory for cutting. As it left the cutter it fell through a chute where it was heated by means of a steam jet to the particular temperature desired. The plan followed was to fill three pairs of vats with cabbage at approximately 42°, 60°, and 70°, respectively. Each pair of vats was filled simultaneously so as to have the same grade of cabbage in both. Of the six vats used in the experiment Nos. 2, 6, 5, and 7 were filled the same day with 9 tons of cabbage and Nos. 3 and 9 were filled a day later with 6 tons.

Three copper-constantan thermocouples were put into each vat; one 2 feet from the bottom and the same distance from the side of the vat, another in the center, and the third 2 feet from the top and a like distance from the outside of the vat, but opposite the bottom thermocouple. The cold junction of the thermocouples was kept in ice water in a Dewar flask and the temperature of the junction in the vat read on a Leeds-Northrup potentiometer graduated directly in degrees Fahrenheit.

Another method of obtaining temperatures was used with four of the vats. A bamboo pole, the intersections of which had been bored out, was placed in the middle of the vat at the time of filling. In this tube were suspended thermometers at heights of 2 feet and 4 feet from the bottom. To prevent a change in the reading while the thermometer was being withdrawn and read the lower end was inclosed in a small tube filled with water.

In order to obtain samples of sauerkraut juice small Büchner funnels were attached to thick-walled rubber tubes and were placed in the vats near the top and bottom thermocouples. The sample was drawn into a receiving bottle by means of a hand-operated suction pump. The first portion of the juice was discarded to insure that the sample used for analysis was obtained from the vat and was not juice that had been standing in the tube. Titratable acidity, number of bacteria, and time of methylene-blue reduction were determined on each sample. The methods used for these determinations, and also for the analysis of the sauerkraut, have been described in a previous publication.[8]

TABLE 2.—*Temperature of sauerkraut in factory vats as determined by thermometers at various times during the fermentation*

Age of sauerkraut (days)	Temperature of factory (° F.)	Temperature (° F.) in—							
		Vat 2		Vat 6		Vat 3		Vat 9	
		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
3	45	43	41	43	41	------	------	57	59
4	48	42	45	42	43	60	54	62	62
6	56	44	46	42	45	62	58	62	63
7	60	44	48	43	45	64	59	64	63
9	50	45	48	44	46	63	58	64	64
10	38	45	48	46	46	62	57	64	65
14	54	48	50	------	50	59	54	------	------
17	55	50	53	52	54	60	57	------	------
23	57	52	53	53	54	61	59	------	------
31	52	53	50	52	52	60	57	------	------
35	55	52	51	52	51	56	53	------	------

[8] PARMELE, H. B., FRED, E. B., PETERSON, W. H., MCCONKIE, J. E., and VAUGHN, W. E. Op. cit.

The results of the observations and the analyses are recorded in the accompanying illustrations and tables. The temperatures obtained by means of thermocouples are given in Figures 1 to 3 and those taken with thermometers are recorded in Table 2. The results in most cases do not differ by more than 1° F. Part of this difference is due to the fact that the paired thermometer and thermocouple were a foot or more apart. Unfortunately, because of a change in operation, the last five readings of the thermocouples were erroneous and had to be discarded.

Fermentation at High Temperatures (67° to 75° F.)

The first sign of fermentation was the evolution of gas. A thick layer of foam formed on the surface of the vat during the first night.

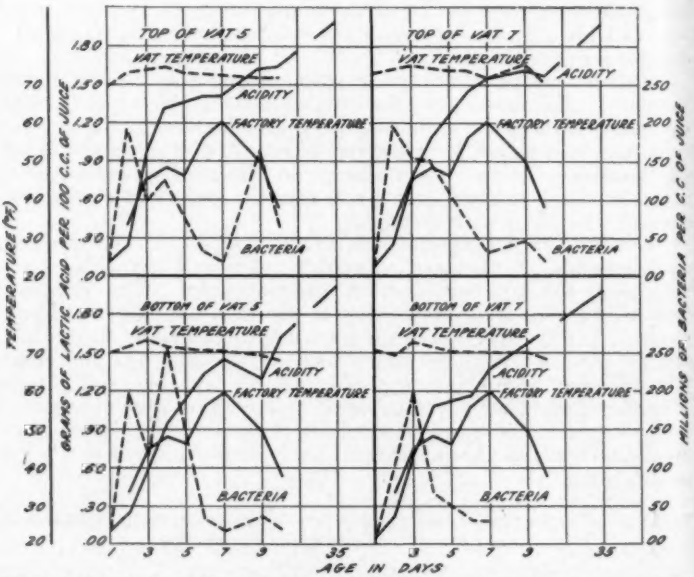

Figure 1.—Temperature, acidity, and number of bacteria in vats 5 and 7 at various times during fermentation of sauerkraut

This did not occur on the vats fermented at a medium temperature until after three days and did not occur at all on the vats fermented at low temperatures.

Figure 1 shows that in general the temperature in vats 5 and 7 rose steadily from the first day to the third or fourth day. This rise in temperature can not be attributed to conduction of heat into the vat from the outside, for the vats were approximately 26° F. above factory temperature at the time they were filled. The production of heat must be caused either by plant-cell respiration or by bacterial activity. The latter was perhaps the most important cause, since the rise in temperature coincided with the time at which the bacteria were present in greatest numbers.

The number of bacteria reached a maximum about the second or third day, which was the time when the acidity began to increase rapidly. This increase was greatest during the third and fourth days. As the acid accumulated the number of bacteria decreased. The type of bacteria also changed after the third day. During the first two or three days coccus forms and short rods predominated, but after three days these gave way to large rods, which persisted throughout the remainder of the fermentation.

Fermentation at Medium Temperatures (57° to 64° F.)

On the first day the temperature in vats 3 and 9 (fig. 2) fell below the initial temperature of the vat. This was probably due to inequalities

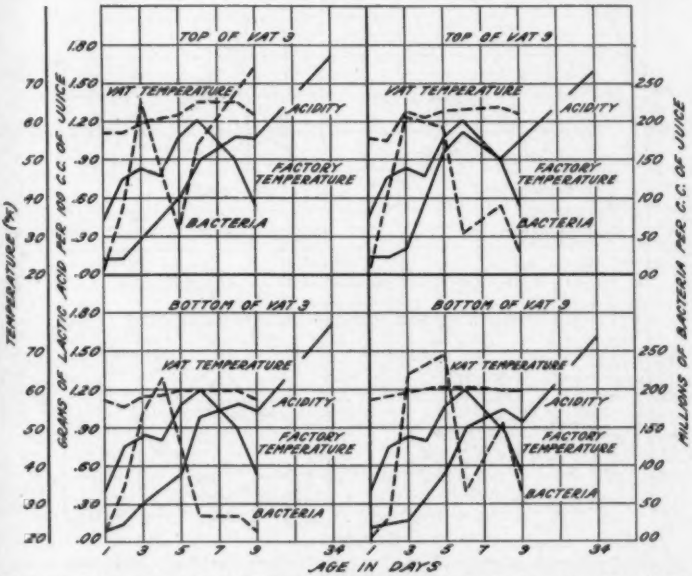

Figure 2.—Temperature, acidity, and number of bacteria in vats 3 and 9 at various times during fermentation of sauerkraut

in the temperature of the cabbage in the vicinity of the thermocouple. The temperature began to rise on the second day and continued to do so for five days after which it remained constant until the eighth day. This rise in temperature may be taken as further evidence of the bacterial origin of the heat developed, for it occurred coincidently with the rise in acidity and the increase in the number of bacteria. If the formation of heat was due to plant-cell respiration the temperature would have risen from the start and would not have been delayed until the second day.

Fermentation at Low Temperatures (40° to 46° F.)

The outstanding facts shown by Figure 3 are that at the low temperature of vats 2 and 6, no appreciable action took place. The gradual rise in temperature was probably the result of heat taken up from the

atmosphere. Acid production was low and the numbers of bacteria were small throughout the fermentation. These results are in agreement with those of experiments previously reported on the production of sauerkraut at low temperatures.[9]

Changes in Time of Methylene-Blue Reduction

The time of methylene-blue reduction may be used as a rough measure of bacterial activity, activity being inversely proportional to the time of reduction, at least approximately. In Table 3 are recorded the changes in the time of methylene-blue reduction of the juice from each of the vats. A comparison of these data with those in

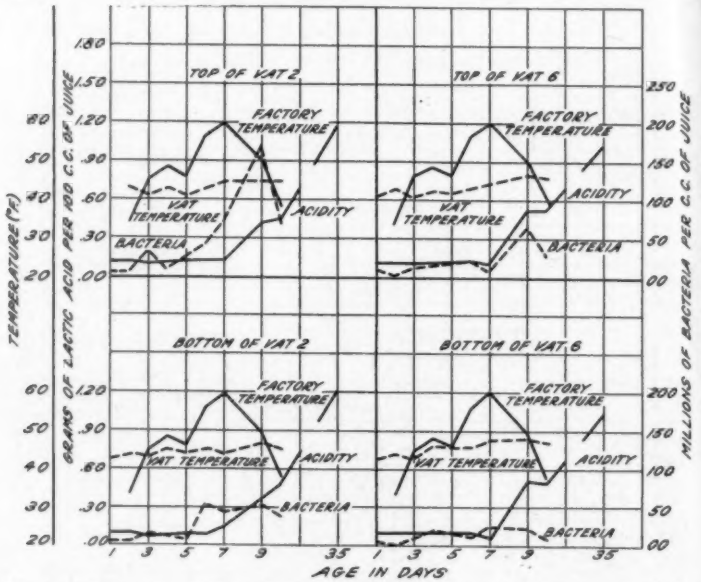

Figure 3.—Temperature, acidity, and number of bacteria in vats 2 and 6 at various times during fermentation of sauerkraut

the illustrations shows that the time of reduction fell from a maximum of over 2 hours at the beginning of the fermentation to a minimum of 15 minutes at the end of 3 to 10 days. The minimum time was reached first by those vats which had the highest initial temperature, i. e., the vats in which fermentation was most active.

Composition and Quality of the Sauerkraut

The vats at high and medium temperatures were opened at the end of 35 days. The low-temperature vats contained so little acid at this time that they were left to ferment and were first opened at the end of 158 days. Samples were taken from the top, the center, and the bottom of each vat. Table 4 gives the results of the analysis and grading.

[9] Priem, L. A., Peterson, W. H., and Fred, E. B. Studies of commercial sauerkraut with special reference to changes in the bacterial flora during fermentation at low temperatures. Jour. Agr. Research 34: 79-95, illus. 1927.

TABLE 3.—*Changes in time of reduction of methylene blue by sauerkraut juice from vats kept at different temperatures during fermentation*

Age of sauerkraut (days)	Time of reduction in minutes for juice from—											
	Vat 2, 42° F.[a]		Vat 6, 42° F.[a]		Vat 3, 57° F.[a]		Vat 9, 57° F.[a]		Vat 5, 70° F.[a]		Vat 7 70° F.[a]	
	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	Top
1	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)
2	(b)	(b)	(b)	(b)	105	(b)	(b)	(b)	80	80	90	90
3	(b)	(b)	(b)	(b)	15	15	15	15	30	30	15	15
4	(b)	(b)	(b)	(b)					15	15	15	15
5					15	15	15	15				
6	105	(b)	(b)	(b)	15	15	15	15	105	15	15	15
7	(b)	(b)	(b)	(b)					15	15	15	15
8					15	15	15	15				
9	60	60	120	90	90	45	45	45	30	30		15
10	15	15	15	90					45	45		60

[a] Initial temperature of fermentation. [b] Not reduced in two hours.

TABLE 4.—*Age, quality, and composition of sauerkraut from experimental vats kept at various fermentation temperatures*

Vat and initial temperature	Age	Moisture	Acetic acid	Lactic acid	Ethyl alcohol	Reducing sugars as glucose	Quality of sauerkraut
	Days	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
Vat 2, 42° F	158	90.5	0.249	1.406	0.282	0.15	Poor.
Vat 6, 42° F	158	89.5	.420	1.576	.326	.12	Do.
Vat 3, 57° F	34	90.2	.371	1.442	.281	.12	Excellent.
Vat 9, 57° F	34	91.9	.332	1.462	.259	.11	Good.
Vat 5, 70° F	35	92.1	.408	1.503	.331	.12	Fair.
Vat 7, 70° F	35	90.6	.420	1.513	.331	.15	Do.

The quality of the sauerkraut which was produced at the highest temperature was only fair judged from the standpoint of flavor, color, and texture. High temperature undoubtedly favors the production of sauerkraut of soft texture and pink color, for numerous soft and pink portions of sauerkraut were found in both vats. It has also been demonstrated in the laboratory that fermentations conducted at high temperatures tend to produce pink sauerkraut. The finest grade of sauerkraut was obtained from the vats kept at intermediate temperatures. The sauerkraut from these vats was white and crisp throughout. The cabbage fermented at the lowest temperature produced sauerkraut that was poor in quality, and possessed a bitter taste, poor texture, and a dark color.

The sauerkraut from the different vats showed no essential difference in percentage content of fermentation products. The quality of sauerkraut depends upon something other than mere acidity. While a proper degree of acidity may be taken as the first requisite, there are other products, as yet not measured, which distinguish a sauerkraut of good flavor from one of poor flavor.

SUMMARY

The quality of sauerkraut was found to depend very largely upon the temperature at which fermentation was carried out, the most favorable temperature for fermentation was between 60° and 65° F.

High temperatures favored the production of soft and pink sauerkraut.

A rise of 3° to 5° F. occurred during the first eight days in fermentation vats kept at temperatures above that of the surrounding air. This rise in temperature was coincident with the greatest activity of the bacteria and is believed to have been caused by bacterial action.

COMPARATIVE VALUE OF THE SIZE OF PHYTOPHTHORA SPORANGIA OBTAINED UNDER STANDARD CONDITIONS[1]

By LEON H. LEONIAN, *Associate Pathologist*, and HERBERT L. GEER, formerly *Assistant in Plant Pathology, West Virginia Agricultural Experiment Station*[2]

INTRODUCTION

Rosenbaum (*25*)[3] was perhaps the first to make a comprehensive effort to evolve unity from the chaotic morphological status of the genus Phytophthora. That his efforts failed materially to influence other workers can be attested by the fact that a large number of so-called new species have been described since the appearance of his paper. This is not at all surprising because a great many investigators still cling to the idea that the species is rigid and immutable. It is perhaps for this same reason that in his work Rosenbaum used only one strain from each species; had he used a dozen different strains, his conclusions would have been remarkably different and his taxonomic key decidedly less simple.

Although the senior writer (*14*) has demonstrated remarkable deviations among the different strains of the same species of Phytophthora affecting shape and size of sporangia, presence and absence of sexual bodies, macroscopic characters of colonies, microscopic nature of submerged hyphae, and even pathogenicity, slight differences have still been used by different investigators as comprehensive bases for the creation of new species.

Since environmental factors exert a great influence upon the morphology of Phytophthoras, and since the size and shape of sporangia constitute the principal basis for the morphological classification of this genus, it becomes obvious that uniform environmental conditions are imperative for dependable measurements. It is not only a useless effort to describe a Phytophthora directly from its host, but it may prove a misleading procedure as well, because a given organism may be described as a different species according to the particular host which it happens to have infected. The complex vegetable media are but little more desirable substrata than the host plant, because not all varieties of oats, carrots, potatoes, plums, and all the heterogeneous constituents of the phytopathologists' agars have the same chemical or physical composition. There can be no dependable morphological classification of Phytophthoras without standardized environmental conditions. But as yet no such uniformity exists, as can be seen from the literature on Phytophthoras. The men who described these organisms worked in different countries, under different climatic and laboratory conditions, and with media that can not be standardized or duplicated. As a result, the literature is teeming with conflicting statements.

[1] Received for publication Mar. 8, 1929, issued August, 1929. Scientific Paper No. 77 of the West Virginia Agricultural Experimental Station.
[2] The writers express their thanks to Russell Brown for his help in biometrical computations.
[3] Reference is made by number (italic) to "Literature cited," p. 309.

SIZES OF SPORANGIA RECORDED IN THE LITERATURE

In order to show the confusion which now exists in the literature, the writers have listed in Table 1 the sizes of sporangia as given by some reprsentative workers with this genus. Even a cursory glance is sufficient to show the amazing lack of consistency throughout the list. But just how much of this is caused by an absence of uniformity in technic and environmental conditions, and how much by the normal differences in the different strains and mutants of the same species, may never be known.

The average size, whenever given, is selected and given in Table 1 as the more comprehensive measure.

TABLE 1.—*Review of sporangial measurements of Phytophthoras as noted by other workers*

Organism	Investigator	Measurements in microns
P. allii	Tanaka (*33*)	36.5 by 49.4.
P. arecae	Coleman (*5*)	20.6 by 30. 45.4 by 51.2. 43.3 by 71.
	Wilson (*38*)	20.6–45.4 by 30.1 by 71.
	Rosenbaum (*25*)	47.92 by 30.05.
	Sundararaman and Ramakrishnan (*32*).	27–42 by 32–64.
	Cohn and Lebert (*25*)	48 by 35–68.
	Schroeter (*25*)	35–40 by 50–60.
	Saccardo (*28, p. 237–238*)	35–40 by 50–90.
	Hartig (*24* and *25*)	25–40 by 25–40.
	Osterwalder (*25*)	14.64–24.4 by 119.56.
	Zimmerman (*25*)	17–30 by 25–60.
	Hori (*25*)	30–50 by 50–60.
	Bubak (*25*)	15–25 by 15–120.
	Van Hook (*25*)	30–42 by 40–58.
P. cactorum	Lafferty and Pethybridge (*10*)	27 by 37 (oatmeal agar). 30 by 52 (raw apple tissue).
	Stevens and Plunkett (*31*)	21–29 by 22–32.
	Rosenbaum (*24*)	27 by 34.5.
	....do	26.06 by 36.62.
	Beach (*1*)	24.9 by 34.4 (apple strain).
	....do. (*1*)	25.97 by 32.34 (first of rhubarb strain).
	....do. (*1*)	27.5 by 37 (second of rhubarb strain).
	....do. (*1*)	25.2 by 33.8 (third of rhubarb strain).
	Rose (*22*)	25.09 by 32.26.
	Rose and Lindegren (*23*)	26.8 by 34.4 (artificial culture). 36.9 by 40.3 (diseased fruits).
Belpharospora cambivora	Petri (*19*)	40–54 by 60–75.
P. capsici	Leonian (*11*)	36 by 60.
P. cinnamomi	Rands (*20*)	33 by 57.
P. citrophthora	Smith and Smith (*29*)	35 by 50.
	Carne (*3*)	19–37 by 19–60 (average 30 by 38.5).
P. colocasiae	Wilson (*38*)	18–26 by 30–60, or larger.
	Butler and Kulkarni (*7*)	18–26 by 38–60.
	McMurphy (*15*)	30–42 by 42–78.
P. erythroseptica	MacMillan (*18*)	20 by 32.
	Wilson (*38*)	20 by 30.
	Rosenbaum (*25*)	27.65 by 44.85.
	Wilson (*38*)	25 by 30, rarely 42 by 80.
	Tucker (*36*)	30.95 by 52.67.
	Reinking (*21*)	31.28 by 52.27.
P. faberi	McRae (*16*)	16–36 by 34–70 (cacao fruit).
	....do	25–32 by 32–66 (Hevea fruits).
	Von Faber (*5*)	25–30 by 42–80.
	Rosenbaum (*25*)	32.29 by 48.52.
	Rosenbaum (*17*)	25.5–43.49 by 37.5–59.49.
	Ocfemia and Roldan (*17*)	29.45 by 44.71.
P. fagi	Wilson (*38*)	15–30 by 30–40.
	Lafferty and Pethybridge (*10*)	31 by 56 (raw apples). 31 by 45 (artificial media).
	Rosenbaum (*25*)	25.67 by 33.65.
P. hibernalis	Carne (*3*)	16.1 by 34.6 (lemon leaves, on potato-dextrose agar). 16.4 by 34.6 (leaf and fruit tissues). 14.3 by 30.3 (potato-dextrose agar).
P. infestans	Saccardo (*26*)	15–20 by 27–30.
	Berlese (*2*)	16–24 by 22–32.
	Clinton (*4*)	11–20 by 17–35.
	Rosenbaum (*25*)	18.27 by 27.08.

TABLE 1.—*Review of sporangial measurements of Phytophthoras as noted by other workers*—Continued

Organism	Investigator	Measurements in microns
P. jatrophe	Rosenbaum (*25*)	37.55 by 49.65.
P. meadii	McRae (*16*)	21 by 48 (Hevea fruits). 30 by 48 (French-bean agar). 23 by 32 (Aqua submersis).
P. melongenae	Tanaka (*33*)	33.9 by 42.4.
P. mexicana	Hotson and Hartge (*9*)	16–33 by 16–77 (artificial culture). 24.2 by 46.2 (tomato fruit).
P. nicotianae	Van Breda de Haan (*35*)	25 by 36.
	Rosenbaum (*25*)	29.95 by 37.58.
	Tisdale and Kelley (*35*)	25.3 by 36.67 (Holland strain). 28.98 by 38.2 (Java strain). 15.75 by 21–38 by 56 (oatmeal agar). 32 by 51.6 (tobacco plant).
P. omnivora	Berlese (*2*)	35 by 50–60.
	Stevens (*30*)	35–40 by 50–60.
	Wilson (*38*)	17–30 by 20–60.
P. parasitica	Dastur (*35*)	20–40 by 25–50.
	Wilson (*38*)	10–45 by 16–60.
	Tisdale and Kelley (*35*)	25.86 by 31.25.
	Rosenbaum (*25*)	23.39 by 43.64.
	Dastur (*6*)	10–15 by 13–20 (Vinca rosea).
	Godfrey (*7*)	21–42 by 27–54.
P. parasitica rhei	Leonian (*15*)	33.2 by 43.7 (Type I). 36.7 by 43.7 (Type II). 37.3 by 43.7 (Type III). 21 by 25.5 (Type IV). 28 by 35 (Type V).
P. phaseoli	Saccardo (*27, p. 341*)	20–24 by 35–50.
	Clinton (*4*)	17–27 by 28–43.
	Rosenbaum (*25*)	19.05 by 27.87.
P. pini	Leonian (*12*)	35 by 55.5.
	Gravatt (*8*)	19–40 by 28–86.
P. syringae	Wilson (*38*)	30–32 by 40–74.
	Rosenbaum (*25*)	25.33 by 39.86.
	Lafferty and Pethybridge (*10*)	26 by 38 (Klebahn's strain). 27 by 40 (apple strain).
P. terrestris	Sherbakoff (*28*)	30.5 by 42.5.
P. thalictri	Wilson (*38*)	13–17 by 20–27.
	Thurston and Orton (*34*)	16.7–22.3 by 20.4–29.7.
P. theobromae	Von Faber (*5*)	25–42 by 30–80.

Twenty-one different measurements are recorded in Table 1 for *Phytophthora cactorum*. Obviously a measurement which reads 15–25 by 15–120 microns is meaningless because almost any organism may be included in such a broad sphere. Saccardo's 35–40 by 50–90 microns, or Schroeter's 35–40 by 50–60 microns are decidedly different from Rosenbaum's measurement of 27 by 34.5 microns, or Beach's 24.9 by 34.5 microns. *P. erythroseptica* recorded by Wilson as measuring 20 by 30 microns materially differs from Rosenbaum's 27.65 by 44.85 microns.

If size is to be used as a dependable factor in taxonomy, such glaring contrasts in the measurements of different investigators must first be eliminated. Standardized conditions whereby all workers use the same simple media, and the same environmental conditions regardless of their localities, should help considerably in the elimination of doubtful factors. With this purpose in mind, the writers undertook to work out the size factor in the organisms listed below.

Information regarding the following group of 48 organisms is given by the first author (*12 pp. 444–447*):

Phytophthora arecae (Colem.) Pethyb.
P. cactorum 1 (Lebert and Cohn) Schrot.
P. cactorum 2 (Beach).
P. cactorum 3.
P. capsici Leonian.

P. citrophthora Smith and Smith.
P. crytogea Pethyb. and Laff.
P. fagi (Hartig) Hartig.
The infestans group:
P. infestans (Mont.) De Bary.
P. phaseoli Thaxter.
P. mexicana Hotson and Hartge.
P. nicotianae Breda de Haan.
The omnivora group:
P. colocasiae Racib.
P. faberi Maubl.
P. palmivora (Butl.) Butl.
P. parasitica Dastur.
P. parasitica rhei I Godfrey.
P. parasitica rhei II.
P. parasitica rhei III.
P. parasitica rhei IV.
P. parasitica rhei V.
P. sp. Reddick's I.
P. terrestris Sherb.
P. pini Leonian—
A collection of tropical Phytophthoras sent by Carl Hartley as follows: 8, 22, 26, 36, 44, 97, 100, 102, 116, 117, 121, 123, 126, 130, 136, 137, 138, 139, 140, 141, 142, 143, 144, and 145.
P. sp. from Byrophyllum; collected and isolated by A. Berg in Bermuda.
P. sp. from tobacco; sent to Leonian by Tisdale.
P. sp. from tomato; isolated by Leonian at Morgantown, W. Va.
P. sp. from Vinca; isolated by Leonian; last material furnished by E. F. Smith.

The foregoing four organisms have been identified as *P. omnivora.*

The following group of organisms was sent by H. S. Fawcett:
No. 760, from tomato: isolated by Sherbakoff.
No. 846, from citrus, southern California.
No. 1026, from orange fruit, Riverside, Calif.
No. 1216, sent from Amsterdam as *P. parasitica.*
No. 1245, from citrus, southern California.
No. 1246.
No. 1274, from citrus, southern California.
No. 1287, from citrus, southern California.
No. 1290 b, from citrus tree, Corsica.
No. 1290 d, "Supposed to be the fungus described by Petri isolated from chestnut trees." *(Blepharospora cambivora.)* This is undoubtedly a Phytophthora.
No. 1305 i, from lemon fruit, Corona, Calif.
No. 1306, from lemon gummosis.
No. 1308 f, from lemon fruit, Riverside, Calif.
No. 1309 a, from lemon gummosis, La Habra, Calif.
No. 1341, from orange fruit, Rivera, Calif.

The following organisms were sent by Tucker:
No. 1, from coconut bud; isolated by Tucker in Porto Rico.
No. 2, from coconut bud; isolated by Reinking in the Philippines; sent to Tucker by Ocfemia.
No. 3, from coconut bud; isolated by Ashby in Jamaica.
No. 4, from cacao pod; isolated by Ashby in Trinidad.
No. 6, from cacao pod; isolated by Roldan in the Philippines, sent to Tucker by Ocfemia.
No. 7, from cacao pod; isolated by Gadd in Ceylon.
No. 8, from cotton boll; isolated by Tucker in Porto Rico.
No. 9, from cotton boll; isolated by Ashby in St. Vincent.
No. 10, from cotton boll; isolated by Wakefield in Montserrat.
No. 11, from cotton boll; isolated by Ashby in Trinidad.
No. 12, from breadfruit; isolated by Gadd in Ceylon.
No. 13, from pawpaw fruit; isolated by Gadd in Ceylon.
No. 16, from Dendrobium Maccarthiae; isolated by Gadd in Ceylon.
No. 161, from eggplant; sent to Tucker by Dreschler who received it from Kendrick.

No. 162, sent to Tucker by Dreschler, labeled "Church 528 Phytophthora Reddick II." Leonian's "Mutant" of Reddick's Phytophthora.
No. 165, from Bermuda lily stump rot; sent to Tucker by Dreschler.
No. 178, from grapefruit; sent to Tucker by Dreschler who received it from Fulton, originally labeled "Grapefruit Phytoph. P. R. 1."
No. 181, from tomato fruit; sent to Tucker by Dreschler who received it from Kendrick, originally labeled "Kendrick 3269."
No. 182, from eggplant; sent to Tucker by Dreschler who received it from Kendrick.
No. 183, from pepper fruit; sent to Tucker by Dreschler who received it from Kendrick, originally labeled "Kendrick 3259."
No. 185, from pineapple, Jamaica; sent to Tucker by Dreschler who received it from Hansford.
No. 186, from tomato fruit; sent to Tucker by Dreschler who received it from Kendrick, originally labeled "Kendrick 3207."
No. 187, from tomato buckeye rot, Washington, D. C.; sent to Tucker by Dreschler, originally labeled "*P. parasitica* var. *lycopersici*."
No. 191, from tomato root; sent to Tucker by Dreschler.
No. 194, from *Lilium candidum;* sent to Tucker by Dreschler.
No. 197, from potato tuber, Kentucky; sent to Tucker by Dreschler who received it from Baarn.
No. 201, from eggplant, Philippines; sent to Tucker by Dreschler who received it from Ocfemia; originally labeled "Eggplant Phytophthora II."
No. 203, from potato tuber, Oklahoma; sent to Tucker by Dreschler.
No. 206, from potato tuber, Idaho; sent to Tucker by Dreschler; labeled "Idaho 969."
No. 207, from potato; sent to Tucker by Dreschler who received it from Welch labeled "*P. infestans;*" not *P. infestans* according to Dreschler; undoubtedly a strain of *P. omnivora.*
No. 213, from eggplant in Philippines; sent to Tucker by Ocfemia; labeled "*P. melongenae;*" this is nothing but *P. ominivora.*

TECHNIC

The writers have followed the method developed by Leonian (*13*), which consists of obtaining a vigorously growing sterile mycelium and allowing it to fruit in a simple solution. The nutrient solution used in this work contains the following ingredients:

Nucleinic acid	1.0 gram.
Dihydrogen potassium phosphate	.6 gram.
Magnesium sulfate	.3 gram.
Dextrose	3.6 grams.
Distilled water	1,000 cubic centimeters.

Twenty-five cubic centimeters of this solution was poured into each of a series of glass capsules (preparation dishes) of 35 cubic centimeters capacity and sterilized. Then a bit of mycelium was transferred to this from the primary-stock cultures carried on oatmeal agar, and allowed to grow. Such colonies constituted the secondary stock cultures from which transfers were made to another series of capsules for the production of sterile mycelia. The cultures were then incubated at 25° C. until a good growth of submerged sterile mycelium was obtained in each dish, a period of three days usually being found sufficient. If allowed to remain longer, the hyphae may reach the surface of the solution and form zoosporangia. The sterile colonies thus obtained were removed from the nutrient solution, washed in sterile distilled water, and transferred to glass capsules each containing 2 cubic centimeters of M/100 solution of potassium nitrate. These cultures were then incubated for three days at 25°, and thereafter examined for zoosporangia. Since only reproduction

and no vegetative growth can take place in potassium nitrate solution, the number of immature sporangia was thus cut down to a minimum.

In most cases 200 representative zoosporangia were measured. Occasionally, when too wide a variation was manifested, as many as 400 measurements were made. On the other hand, when the zoosporangia were too scanty in number or when they exhibited an apparently close uniformity in size, only 100 sporangia were measured.

The sporangia of *Phytophthora infestans*, *P. phaseoli*, and *P. thalictri* were taken directly from their respective hosts and measured because of the difficulty of growing these three organisms on artificial media.

TABLE 2.—*Length and width dimensions of the sporangia expressed in microns*

Phytophthora organism	Length		Width	
	Mean	Mode	Mean	Mode
P. arecae	34.52±0.422	37.0	24.67±0.222	25.9
P. cactorum, 1	32.67± .443	33.3	27.49± .292	29.6
P. cucturum, 2	36.48± .451	33.3	31.82± .310	33.3
P. cactorum, 3	39.55± .648	37.0	28.89± .315	29.6
P. capsici	44.51± .838	40.7	29.89± .371	29.6
P. citrophthora	49.17± .860	51.8	30.96± .321	31.3
P. fagi	36.22± .532	37.0	28.97± .289	29.6
P. infestans	30.08± .382	27.7	18.90± .165	18.5
P. phaseoli	34.81± .270	37.0	20.09± .170	18.5
P. mexicana	50.20± .804	51.8	32.93± .379	29.6
P. colocasiae	37.70± .909	37.0	24.05± .404	25.9
P. faberi	39.66± .533	37.0	30.63± .331	29.6
P. palmivora	39.18± .490	37.0	26.67± .194	25.9
P. parasitica	40.03± .608	44.4	31.80± .419	33.3
P. parasitica rhei, I	40.77± .690	40.7	30.11± .428	−25.9
P. parasitica rhei, II	40.14± .639	44.4	30.82± .424	33.3
P. parasitica rhei, IV	27.01± .332	24.5	25.93± .354	22.2
P. sp. (Reddick), I	43.36± .978	37.0	30.52± .473	29.6
P. sp. (Reddick), II	46.80± .788	48.1	36.03± .536	37.0
P. terrestris	41.03± .565	44.4	32.15± .366	37.0
P. pini	43.25± .518	37.0	28.78± .282	29.6
P. thalictri	20.60± .187	22.2	15.76± .168	14.8
P. sp. 8 (Hartley)	33.52± .574	29.6	25.38± .287	25.9
P. sp. 22 (Hartley)	42.62± .556	44.4	30.52± .361	29.6
P. sp. 26 (Hartley)	38.62± .598	37.0	30.08± .412	29.6
P. sp. 36 (Hartley)	36.03± .435	37.0	28.56± .318	29.6
P. sp. 44 (Hartley)	35.66± .413	37.0	28.23± .307	25.9
P. sp. 97 (Hartley)	40.81± .781	33.3	28.34± .285	25.9
P. sp. 100 (Hartley)	35.66± .546	37.0	28.60± .282	29.6
P. sp. 102 (Hartley)	38.40± .623	37.0	29.89± .319	29.6
P. sp. 116 (Hartley)	41.81± .725	37.0	26.93± .271	25.9
P. sp. 117 (Hartley)	41.84± .605	37.0	31.22± .348	33.3
P. sp. 121 (Hartley)	40.03± .545	37.0	30.11± .295	29.6
P. sp. 123 (Hartley)	40.73± .894	37.0	33.59± .512	33.3
P. sp. 126 (Hartley)	36.29± .486	37.0	29.93± .322	25.9
P. sp. 130 (Hartley)	37.29± .464	35.1	28.37± .282	29.6
P. sp. 136 (Hartley)	37.44± .412	37.0	27.97± .235	29.6
P. sp. 137 (Hartley)	36.26± .562	37.0	28.86± .329	29.6
P. sp. 138 (Hartley)	44.51± .601	48.1	32.00± .359	37.0
P. sp. 140 (Hartley)	39.70± .590	37.0	32.37± .443	29.6
P. sp. 141 (Hartley)	36.77± .469	33.3	29.41± .443	29.6
P. sp. 142 (Hartley)	36.88± .751	29.6	28.78± .336	29.6
P. sp. 143 (Hartley)	33.44± .462	29.6	29.74± .340	29.6
P. sp. 144 (Hartley)	32.59± .420	37.0	27.04± .299	29.6
P. sp. 145 (Hartley)	44.06±1.402	33.3	30.30± .303	29.6
P. sp. (on Bryophilum)	36.33± .663	33.3	30.26± .420	29.6
P. sp. (on tobacco)	39.88± .611	33.3	30.45± .451	29.6
P. sp. (on tomato)	31.22± .515	25.9	27.01± .363	25.9
P. sp. (on Vinca)	38.03± .587	40.7	29.78± .389	29.6
P. sp. 760 (Fawcett)	37.85± .551	37.0	30.19± .358	29.6
P. sp. 846 (Fawcett)	47.36± .683	44.4	28.63± .214	29.6
P. sp. 1026 (Fawcett)	43.30± .498	40.7	29.48± .265	29.6
P. sp. 1216 (Fawcett)	34.85± .548	31.3	25.45± .341	25.9
P. sp. 1245 (Fawcett)	45.17± .392	48.1	29.78± .230	29.6
P. sp. 1246 (Fawcett)	38.07± .577	37.0	25.56± .264	25.9
P. sp. 1274 (Fawcett)	41.10± .390	37.0	24.34± .191	25.9
P. sp. 1287 (Fawcett)	51.54± .655	50.0	30.45± .252	29.6

TABLE 2.—*Length and width dimensions of the sporangia expressed in microns*—Continued

Phytophthora organism	Length		Width	
	Mean	Mode	Mean	Mode
P. sp. 1290b (Fawcett)	49. 54±1. 140	37. 0	30. 81± . 336	29. 6
P. sp. 1290d (Fawcett)	37. 44± . 564	29. 6	23. 71± . 367	22. 2
P. sp. 1305i (Fawcett)	43. 32± . 539	44. 4	30. 15± . 288	29. 6
P. sp. 1306 (Fawcett)	52. 45±1. 381	44. 4	30. 56± . 364	29. 6
P. sp. 1308F (Fawcett)	46. 32± . 687	44. 4	30. 08± . 295	29. 6
P. sp. 1309a (Fawcett)	50. 50± . 909	48. 1	28. 67± . 346	29. 6
P. sp. 1341 (Fawcett)	49. 35± . 803	48. 1	31. 33± . 359	33. 3
P. sp. 1 (Tucker)	39. 40± . 475	37. 0	28. 23± . 312	25. 9
P. sp. 2 (Tucker)	35 92± . 432	33. 3	30. 00± . 317	29. 6
P. sp. 3 (Tucker)	42. 14± . 541	44. 4	27. 15± . 235	29. 6
P. sp. 4 (Tucker)	38. 85± . 457	37. 2	32. 15± . 361	29. 6
P. sp. 6 (Tucker)	46. 95± . 802	48. 1	31. 74± . 353	29. 6
P. sp. 7 (Tucker)	40. 62± . 441	44. 4	32. 30± . 353	33. 3
P. sp. 8 (Tucker)	43. 51± . 561	46. 2	36. 70± . 336	40. 7
P. sp. 9 (Tucker)	48. 35± . 674	51. 8	29. 04± . 296	29. 6
P. sp. 10 (Tucker)	49. 61± . 589	48. 1	40. 44± . 469	40. 7
P. sp. 11 (Tucker)	48. 65± . 977	44. 4	32. 74± . 281	29. 6
P. sp. 12 (Tucker)	36. 55± . 348	37. 0	28. 12± . 266	25. 9
P. sp. 13 (Tucker)	39. 50± . 507	44. 4	29. 34± . 333	33. 3
P. sp. 16 (Tucker)	40. 25± . 518	37. 0	28. 93± . 260	29. 6
P. sp. 161 (Tucker)	39. 92± . 576	40. 7	30. 04± . 440	29. 6
P. sp. 165 (Tucker)	40. 33± . 606	33. 3	33. 33± . 473	29. 6
P. sp. 178 (Tucker)	50. 13± . 906	48. 1	34. 11± . 458	29. 6
P. sp. 181 (Tucker)	48. 50± . 876	40. 7	32. 89± . 363	29. 6
P. sp. 182 (Tucker)	40. 99± . 906	29. 6	31. 22± . 452	29. 6
P. sp. 183 (Tucker)	40. 7± . 807	37. 0	30. 93± . 482	29. 6
P. sp. 185 (Tucker)	43. 10± . 586	44. 4	30. 96± . 330	33. 3
P. sp. 186 (Tucker)	39. 88± . 587	40. 7	31. 70± . 326	29. 6
P. sp. 187 (Tucker)	41. 88± . 441	44. 4	34. 07± . 371	37. 0
P. sp. 191 (Tucker)	40. 62± . 766	33. 3	32. 56± . 467	29. 6
P. sp. 194 (Tucker)	32. 85± . 279	33. 3	26. 49± . 205	25. 9
P. sp. 197 (Tucker)	44. 58± . 661	48. 1	32. 39± . 364	37. 0
P. sp. 201 (Tucker)	38. 99± . 581	37. 0	31. 22± . 386	29. 6
P. sp. 203 (Tucker)	46. 95±1. 189	29. 6	30. 74± . 489	25. 9
P. sp. 206 (Tucker)	42. 14± . 420	38. 8	27. 13± . 173	37. 0
P. sp. 207 (Tucker)	44. 95± . 650	44. 4	36. 44± . 379	37. 0
P. sp. 213 (Tucker)	36. 40± . 382	37. 0	28. 30± . 281	25. 9

RESULTS

The results of the measurements made by the writers appear in Table 2. The authors have checked each other's measurements to make certain that no unconscious selection was practiced and that errors caused by the personal factor were reduced to a minimum. While the measurements checked fairly closely, it is recognized that the measurements of zoosporangia can never be highly standardized nor confined to the limits of mathematical pigeonholes.

The length of sporangia arranged according to the arbitrary size limits of 20–25 microns, 28–33 microns, 36–40 microns, 43–48 microns, and 50 microns and over, yields five groups, A, B, C, D, and E as shown in Table 3.

TABLE 3.—*Grouping of Phytophthoras according to the mean length of sporangia*

Group A, 20 to 25 microns	Group B, 28 to 33 microns	Group C, 36 to 40 microns	Group D, 43 to 48 microns	Group E, 50 microns and over
P. thalictri.	P. arecae. P. cactorum, I. P. infestans. P. phascoli. P. parasitica rhei, IV. P. sp. 8 (Hartley). P. sp. 143 (Hartley). P. sp. 144 (Hartley). P. sp. (tomato). P. sp. 1216 (Fawcett). P. sp. 194 (Tucker).	P. cactorum, 2. P. cactorum, 3. P. fagi. P. colocasiae. P. faberi. P. palmivora. P. parasitica. P. parasitica rhei, I. P. parasitica rhei, II. P. terrestris. P. sp. 26 (Hartley). P. sp. 36 (Hartley). P. sp. 44 (Hartley). P. sp. 97 (Hartley). P. sp. 100 (Hartley). P. sp. 102 (Hartley). P. sp. 121 (Hartley). P. sp. 123 (Hartley). P. sp. 126 (Hartley). P. sp. 130 (Hartley). P. sp. 136 (Hartley). P. sp. 137 (Hartley). P. sp. 139 (Hartley). P. sp. 140 (Hartley). P. sp. 141 (Hartley). P. sp. 142 (Hartley). P. sp. (Bryophilum). P. sp. (tobacco). P. sp. (Vinca). P. sp. 760 (Fawcett). P. sp. 1246 (Fawcett). P. sp. 1274 (Fawcett). P. sp. 1290 d (Fawcett). P. sp. 1 (Tucker). P. sp. 2 (Tucker). P. sp. 4 (Tucker). P. sp. 7 (Tucker). P. sp. 12 (Tucker). P. sp. 13 (Tucker). P. sp. 16 (Tucker). P. sp. 161 (Tucker). P. sp. 165 (Tucker). P. sp. 182 (Tucker). P. sp. 183 (Tucker). P. sp. 186 (Tucker). P. sp. 191 (Tucker). P. sp. 201 (Tucker). P. sp. 213 (Tucker).	P. capsici. P. citrophthora. P. sp. (Reddick), I. P. sp. (Reddick), II. P. pini. P. sp., 22 (Hartley). P. sp. 116 (Hartley). P. sp, 117 (Hartley). P. sp. 138 (Hartley). P. sp. 145 (Hartley). P. sp. 846 (Fawcett). P. sp. 1026 (Fawcett). P. sp. 1245 (Fawcett). P. sp. 1305 (Fawcett). P. sp. 1308 (Fawcett). P. sp. 3 (Tucker). P. sp. 6 (Tucker). P. sp. 8 (Tucker). P. sp. 9 (Tucker). P. sp. 10 (Tucker). P. sp. 11 (Tucker). P. sp. 181 (Tucker). P. sp. 185 (Tucker). P. sp. 187 (Tucker). P. sp. 197 (Tucker). P. sp. 203 (Tucker). P. sp. 206 (Tucker). P. sp. 207 (Tucker).	P. mexicana. P. sp. 1287 (Fawcett). P. sp. 1290 b (Fawcett). P. sp. 1306(Fawcett). P. sp. 1309(Fawcett). P. sp. 1341 (Fawcett) P. sp. 178 (Tucker).

The foregoing groups can by no means be considered stable, as Group A may easily merge with the lower limits of Group B, although not always so readily with those of C; similarly Group B may merge with the lower limits of C more frequently than with those of D, and so on. Group C, characterized by a size factor of 36–40 microns, contains the majority of individuals, followed by the next group which is distinguished by a length of 43–48 microns.

If grouped according to the width of sporangia, and according to the following arbitrary sizes of 18–22 microns, 25–28 microns, 31–34 microns, and 36 microns and over, four groups can be formed as shown in Table 4.

TABLE 4.—*Grouping of Phytophthoras according to the mean width of sporangia*

Group A, 18 to 22 microns	Group B, 25 to 28 microns	Group C, 31 to 34 microns	Group D, 36 microns and over
P. infestans.	P. arecae.	P. cactorum 2.	P. sp. 8 (Tucker).
P. phaseoli.	P. cactorum, 1.	P. citrophthora.	P. sp. 10 (Tucker).
P. thalictri.	P. cactorum, 3.	P. mexicana.	P. sp. 162 (Tucker).
P. sp. 1290d (Fawcett).	P. capsici.	P. faberi.	P. sp. 207 (Tucker).
	P. fagi.	P. parasitica.	
	P. colocasiae.	P. parasitica rhei, I.	
	P. palmivora.	P. parasitica rhei, II.	
	P. parasitica rhei, IV.	P. sp. (Reddick).	
	P. pini.	P. terrestris.	
	P. sp. 8 (Hartley).	P. sp. 22 (Hartley).	
	P. sp. 36 (Hartley).	P. sp. 26 (Hartley).	
	P. sp. 44 (Hartley).	P. sp. 117 (Hartley.)	
	P. sp. 97 (Hartley).	P. sp. 121 (Hartley).	
	P. sp. 100 (Hartley).	P. sp. 123 (Hartley).	
	P. sp. 102 (Hartley).	P. sp. 138 (Hartley).	
	P. sp. 116 (Hartley).	P. sp. 139 (Hartley).	
	P. sp. 126 (Hartley).	P. sp. 140 (Hartley).	
	P. sp. 130 (Hartley).	P. sp. 145 (Hartley).	
	P. sp. 136 (Hartley).	P. sp. (Bryophilum).	
	P. sp. 137 (Hartley).	P. sp. (tobacco).	
	P. sp. 141 (Hartley).	P. sp. 760 (Fawcett).	
	P. sp. 142 (Hartley).	P. sp. 1287 (Fawcett).	
	P. sp. 143 (Bartley).	P. sp. 1290b (Fawcewtt).	
	P. sp. 144.	P. sp. 1305b (Fawcett).	
	P. sp. tomato.	P. sp. 1306b (Fawcett).	
	P. sp. (Vinca).	P. sp. 1308b (Fawcett).	
	P. sp. 846 (Fawcett).	P. sp. 1341b (Fawcett).	
	P. sp. 1026 (Fawcett).	P. sp. 2 (Tucker).	
	P. sp. 1216 (Fawcett).	P. sp. 4 (Tucker).	
	P. sp. 1245 (Fawcett).	P. sp. 6 (Tucker).	
	P. sp. 1246 (Fawcett).	P. sp. 7 (Tucker).	
	P. sp. 1274 (Fawcett).	P. sp. 8 (Tucker).	
	P. sp. 1302a (Fawcett).	P. sp. 10 (Tucker).	
	P. sp. 1 (Tucker).	P. sp. 11 (Tucker).	
	P. sp. 3 (Tucker).	P. sp. 161 (Tucker).	
	P. sp. 9 (Tucker).	P. sp. 162 (Tucker).	
	P. sp. 12 (Tucker).	P. sp. 165 (Tucker).	
	P. sp. 13 (Tucker).	P. sp. 178 (Tucker).	
	P. sp. 16 (Tucker).	P. sp. 181 (Tucker).	
	P. sp. 94 (Tucker).	P. sp. 182 (Tucker).	
	P. sp. 206 (Tucker).	P. sp. 183 (Tucker).	
	P. sp. 213 (Tucker).	P. sp. 185 (Tucker).	
		P. sp. 186 (Tucker).	
		P. sp. 187 (Tucker).	
		P. sp. 191 (Tucker).	
		P. sp. 197 (Tucker).	
		P. sp. 201 (Tucker).	
		P. sp. 203 (Tucker).	
		P. sp. 207 (Tucker).	

The apparent or real kinship of the various organisms becomes still more pronounced where individual strains possessing identical lengths or widths are grouped together as in Table 5.

TABLE 5.—*Grouping together of Phytophthoras with identical length and width of sporangia*

Length	Organism	Width	Organism
Microns		*Microns*	
20	P. thalictri.	15	P. thalictri.
27	P. parasitica rhei, IV.	18	P. infestans.
30	P. infestans.	20	P. phaseoli.
31	P. sp. (tomato).	23	P. sp. 1290d (Fawcett).
32	P. cactorum, 1.	24	P. arecae.
	P. sp. 144 (Hartley).		P. colocasiae.
	P. sp. 194 (Tucker).		P. sp. 1274 (Fawcett).

TABLE 5.—*Grouping together of Phytophthoras with identical length and width of sporangia*—Continued

Length	Organism
Microns	
33	P. sp. 8 (Hartley).
	P. sp. 143 (Hartley).
34	P. arecae.
	P. phaseoli.
	P. sp. 1216 (Fawcett).
35	P. sp. 44 (Hartley).
	P. sp. 100 (Hartley).
	P. sp. 2 (Tucker).
36	P. cactorum 2.
	P. fagi.
	P. sp. 36 (Hartley).
	P. sp. 126 (Hartley).
	P. sp. 137 (Hartley).
	P. sp. 141 (Hartley).
	P. sp. 142 (Hartley).
37	P. colocasiae.
	P. sp. 130 (Hartley).
	P. sp. 136 (Hartley).
	P. sp. 760 (Fawcett).
	P. sp. 1290d (Fawcett).
	P. sp. 4 (Tucker).
	P. sp. 201 (Tucker).
38	P. sp. 26 (Hartley).
	P. sp. 102 (Hartley).
	P. sp. (Vinca).
	P. sp. 1246 (Fawcett).
	P. sp. 4 (Tucker).
	P. sp. 201 (Tucker).
39	P. cactorum, 3.
	P. faberi.
	P. palmivora.
	P. sp. 139 (Hartley).
	P. sp. 140 (Hartley).
	P. sp. (tobacco).
	P. sp. 1 (Tucker).
	P. sp. 13 (Tucker).
	P. sp. 161 (Tucker).
	P. sp. 186 (Tucker).
40	P. parasitica.
	P. parasitica rhei, I.
	P. parasitica rhei, II.
	P. sp. 97 (Hartley).
	P. sp. 121 (Hartley).
	P. sp. 123 (Hartley).
	P. sp. 7 (Tucker).
	P. sp. 16 (Tucker).
	P. sp. 165 (Tucker).
	P. sp. 182 (Tucker).
	P. sp. 191 (Tucker).
41	P. terrestris.
	P. sp. 116 (Hartley).
	P. sp. 117 (Hartley).
	P. sp. 1274 (Hartley).
	P. sp. 187 (Tucker).
43	P. sp. (Reddick) I.
	P. pini.
	P. sp. 1026 (Fawcett).
	P. sp. 1305 (Fawcett).
	P. sp. 8 (Tucker).
	P. sp. 185 (Tucker.)
44	P. capsici.
	P. sp. 138 (Hartley).
	P. sp. 145 (Hartley).
	P. sp. 197 (Tucker).
	P. sp. 207 (Tucker).
45	P. sp. 1245 (Fawcett).
46	P. sp. (Reddick) II.
	P. sp. 1306 (Fawcett).
	P. sp. 6 (Tucker).
	P. sp. 203 (Tucker).

Width	Organism
Microns	
25	P. parasitica rhei, IV.
	P. sp. 8 (Hartley).
	P. sp. 1216 (Fawcett).
	P. sp. 1246 (Fawcett).
26	P. palmivora.
	P. sp. 116 (Hartley).
	P. sp. 194 (Tucker).
27	P. cactorum, 1.
	P. sp. 136 (Hartley).
	P. sp. 144 (Hartley).
	P. sp. (tomato).
	P. sp. 3 (Tucker).
	P. sp. 206 (Tucker).
28	P. cactorum, 3.
	P. fagi.
	P. pini.
	P. sp. 36 (Hartley).
	P. sp. 44 (Hartley).
	P. sp. 97 (Hartley).
	P. sp. 100 (Hartley).
	P. sp. 130 (Hartley).
	P. sp. 137 (Hartley).
	P. sp. 142 (Hartley).
	P. sp. 846 (Fawcett).
	P. sp. 1309 (Fawcett).
	P. sp. 1 (Tucker).
	P. sp. 12 (Tucker).
	P. sp. 16 (Tucker).
	P. sp. 213 (Tucker.)
29	P. capsici.
	P. sp. 102 (Hartley).
	P. sp. 126 (Hartley).
	P. sp. 141 (Hartley).
	P. sp. 143 (Hartley).
	P. sp. (Vinca).
	P. sp. 1026 (Fawcett).
	P. sp. 1245 (Fawcett).
	P. sp. 9 (Tucker).
	P. sp. 13 (Tucker).
30	P. citrophthora.
	P. faberi.
	P. parasitica rhei, I.
	P. parasitica rhei, II.
	P. sp. (Reddick), I.
	P. sp. 22 (Hartley).
	P. sp. 26 (Hartley).
	P. sp. 121 (Hartley).
	P. sp. 145 (Hartley).
	P. sp. (Bryophilum).
	P. sp. (Tobacco).
	P. sp. 760 (Fawcett).
	P. sp. 1287 (Fawcett).
	P. sp. 1290b (Fawcett).
	P. sp. 1305 (Fawcett).
	P. sp. 1306 (Fawcett).
	P. sp. 1308 (Fawcett).
	P. sp. 2 (Tucker).
	P. sp. 161 (Tucker).
	P. sp. 183 (Tucker).
	P. sp. 185 (Tucker).
	P. sp. 203 (Tucker).
31	P. cactorum 2.
	P. parasitica.
	P. sp. 117 (Hartley).
	P. sp. 139 (Hartley).
	P. sp. 1341 (Fawcett).
	P. sp. 6 (Tucker).
	P. sp. 182 (Tucker).
	P. sp. 186 (Tucker).
	P. sp. 201 (Tucker).

TABLE 5.—*Grouping together of Phytophthoras with identical length and width of sporangia*—Continued

Length	Organism	Width	Organism
Microns		*Microns*	
47	P. sp. 846 (Fawcett).		P. mexicana.
	P. sp. 9 (Tucker).		P. terrestris.
48	P. sp. 11 (Tucker).		P. sp. 132 (Hartley).
	P. sp. 181 (Tucker).		P. sp. 140 (Hartley).
	P. citrophthora.		P. sp. 1 (Tucker).
49	P. sp. 1290b (Fawcett).	32	P. sp. 4 (Tucker).
	P. sp. 1341 (Fawcett).		P. sp. 7 (Tucker).
	P. sp. 10 (Tucker).		P. sp. 181 (Tucker).
	P. mexicana.		P. sp. 191 (Tucker).
50	P. sp. 1309a (Fawcett).		P. sp. 197 (Tucker).
	P. sp. 178 (Tucker).	33	P. sp. 123 (Hartley).
51	P. sp. 1287 (Fawcett).		P. sp. 165 (Tucker).
52	P. sp. 1306 (Fawcett).	34	P. sp. 178 (Tucker).
			P. sp. 187 (Tucker).
			P. sp. 8 (Tucker).
		36	P. sp. 207 (Tucker).
			P. sp. (Reddick) II.
		40	P. sp. 10 (Tucker).

DISCUSSION OF RESULTS

It is very evident that similarity or dissimilarity of sporangial sizes does not, necessarily, denote taxonomic kinship. Whereas in reality *Phytophthora thalictri* should be nothing more than a variety of *P. infestans*, it was found that from the standpoint of sporangial sizes alone it is much closer to *P. parasitica* var. *rhei IV*. *P. arecae* and *P. phaseoli* are decidedly different species, yet they are almost identical in so far as the size of their zoosporangia is concerned. There are 28 organisms (Table 5) between *P. cactorum 1* and *P. cactorum 3* if the length of sporangia is considered, and only 8 organisms between *P. cactorum 1* and *P. cactorum 2*, whereas, from the standpoint of the width of zoosporangia, it stands next to *P. cactorum 3* and is 48 organisms removed from *P. cactorum 2*. If three strains of this one species alone show such sharp dissimilarities in the size of their sporangia, despite the rigidly controlled environmental conditions, it is natural that glaring discrepancies are to be noted throughout the taxonomic literature on this and other species of Phytophthoras. Similarly, if some of the strains of *P. parasitica rhei* which were dissociated under the very eye of the senior writer, exceed all reasonable limits of fluctuation and yield decidedly different looking individuals, it is not surprising that so many of the different strains of *P. omnivora* have been described as different species. Arranged according to the mean size of their zoosporangia the following 15 strains of *P. omnivora* manifest a remarkable gradation with a maximum difference of 22 microns between the mean length of *P. parasitica rhei IV* and *P. sp.* 10 (Tucker), and a difference of 15 microns between their mean width, not to say anything about all kinds of intergradations:

	Microns		Microns
P. parasitica rhei IV	27×25	*P. parasitica* (Dastur's strain)	40×31
P. sp. (tomato)	31×27	*P. terrestris*	41×31
P. sp. 144	32×27	P. sp. (Reddick) I	43×30
P. sp. 143	33×29	P. sp. 145	44×30
P. sp. 126	36×29	P. sp. 138	44×32
P. sp. (Vinca)	38×29	P. sp. (Reddick) II	46×36
P. sp. 139	39×31	P. sp. 10 (Tucker)	49×40
P. parasitica rhei I	40×30		

Confronted by such a situation, and with no aid forthcoming from the available taxonomic literature, the worker is obliged to create many new species and varieties in order properly to dispose of such organisms. Yet the anomaly of such a procedure is at once apparent, because the writers know that at least in some cases the so-called new forms sprang from the old as dissociative phases and represent nothing but a stage in the life of a given organism rather than true mutants or immutable entities.

The size of oogonia or oospores is no more dependable for purposes of classification than that of sporangia. The following figures (in microns) for the oogonia of *Phytophthora cactorum*, as given by different authorities, speak for themselves:

16×24, DeBary
22×32, Stevens and Plunkett.
26.78, Rosenbaum, for Phyllocactus strain.
27.36, Rosenbaum, for Panax strain.
26 to 28, Hori.
24 to 30, Schroeter.
20 to 70, Lebert and Cohn.

Because the two strains of *Phytophthora cactorum* which Rosenbaum studied averaged about 27 microns for the oospores one is not justified in assuming that the measurements of other workers were faulty. While it is true that the scheme of computing measurements and the environmental conditions utilized by any given investigator unquestionably exert a material influence upon the size factor, the innate variability and the specific or hereditary peculiarities of different strains may also be responsible for the obvious discrepancies noted in the foregoing figures. Even if 27 microns are accepted as representing the average size of the oospores of *P. cactorum*, one is still confronted by the existence of numerous other species the sexually formed spores of which measure not so very differently from those of *P. cactorum*. Consider, for example, the following measurements in microns:

P. colocasiae	20 to 28.	*P. phaseoli*	25.
P. erythroseptica	29 to 30 (MacMillan).	*P. syringae*	29.5 (Rosenbaum).
P. erythroseptica	35.78 (Rosenbaum).	*P. syringae*	25 (Lafferty and Pethybridge).
P. fagi	20 to 30.	*P. theobromae*	22 to 45.
P. omnivora	24 to 30.	*P. hibernalis*	22 to 45.
P. parasitica rhei	24.	*P. meadii*	16 to 32.

The differences which seem to exist in the size of oospores of different organisms are not large enough for such a fluctuating group of fungi to justify their employment in the primary differentiation of species. When a difference of 5 microns exists in the measurements of Lafferty and Pethybridge and those of Rosenbaum as given in the case of *Phytophthora erythroseptica*, it becomes rather difficult to accept a difference of 2 or 3 microns as of sufficient significance to be employed in taxonomy. Furthermore, since no uniformity of environmental conditions can be found described throughout the literature concerning oospore production and measurements, these figures, as they now exist, possess no value, and merely create confusion. Before the size of oospores can have any value for classification, all work must be repeated under standardized conditions, and at least 20 different strains of each species of Phytophthora should be used before figures of comparative reliance can be obtained.

Furthermore the relation of antheridia to oogonia is not of much significance taxonomically. There are at least four species, *Phytophthora cactorum*, *P. fagi*, *P. syringae*, and *P. pini* in which both para-

gynous and amphigynous antheridia are present. It is highly probable that additional species, if studied intensively enough, may show similar relationships.

The presence or absence of the sexual bodies is not a safe criterion in taxonomy. Aside from the possible occurrence of heterothallism, which has already been demonstrated in some strains of *Phytophthora faberi*, there is the further probability of cyclogenic successions in the life of a given fungus, whereby sexual and sexless phases may alternate irrespective of environmental conditions. Such instances are by no means rare in some strains of *P. infestans* where a fairly large number of oogonia may sometimes develop in oatmeal-agar tubes, and then, upon subsequent transfers, such bodies may altogether disappear for months at a time, no matter how often nor under what conditions subcultures are made. Then, just as suddenly, these bodies may reappear. *P. thalictri* is described by Wilson as possessing no oogonia; Davis failed to find such bodies; Clinton discovered very few in the specimens collected by him; but the strain which was collected in West Virginia and put in pure culture by Berg, forms oogonia in great abundance. Some strains of other species may form sexual bodies very readily, while others may not yield a single oogonium; or they may yield oogonia in culture for a while, and then cease producing them altogether. The senior writer has shown that the so-called mutants developing from a monosporangious culture of *P. parasitica rhei*, manifested some sharp differences in their ability to form sexual bodies. Strains I and II produced oogonia abundantly, Strain V less readily, Strain IV sparingly, and Strain III none at all. Yet these five strains were not mutants, although at first presented as such, but merely different phases in the cyclogeny of the same organism; after breeding true for over three years they reverted to their prototype, and all efforts again to induce segregation met with failure.[2]

Dissociation among fungi is of much greater significance than has hitherto been realized. Many, if not all, of the so-called mutants, saltants, variants, etc., are probably nothing more than dissociative phases in the life of the same fungus. It is difficult to conceive of an organism which will permanently lose or gain certain characteristics, as the adherents of the mutation theory would lead us to believe. If the environment can not put something in the protoplasm which was not there before, how can the same environment remove something that was there? All that environment can do is to cause the emergence or submergence of certain characters. Such being the case, it would be unwise to accept the assumption that the so-called new strains produced by the operation of the mutation phenomenon are specific organisms rather than merely so many phases or facets of one complex organisms. It has been suggested that because the media employed by the senior writer have produced so many dissociations among Phytophthoras and Fusaria, the employment of substrata rich in sugars and organic nitrogen should be avoided. But would not this merely be ignoring truth simply because it happened adversely to affect the smooth working of our taxonomic mechanism?

[2] Just before this paper went to the printer, some further work was undertaken with *P. parasitica rhei* and it was found that after a quiescent period of more than two years this organism began to dissociate once more, and that whereas it formerly produced only 5 strains, now there are nearly 20 of them, with no stability of growth characters in sight. It is probable that additional strains may appear and be isolated in the near future.

STATUS OF DIFFERENT SPECIES

In the light of the foregoing tables and discussions, what can be the status of different species of Phytophthora? While the writers have not had the opportunity of studying a large number of strains of all the species of this genus, and consequently feel rather reluctant to make too many decisive statements, it does seem that at least some of the species are well enough established to withstand future taxonomic upheavals. Others, however, have very little or no secure foundation and should be merged with the older species. The *infestans* group is decidedly secure with its 3 species, *P. infestans*, *P. thalictri*, and *P. phaseoli*. These 3 organisms are very readily distinguished from all others, not only by their morphological features, but especially by the difficulty with which they grow on synthetic media. Whereas all other forms of Phytophthoras studied by the writers make fairly large colonies on malt-extract agar within six days, these 3 species show not the slightest growth in that time. Their host relationship is very narrow and specialized, and their sporangia are among the smallest. Arranged according to the size of these bodies, from the smallest to the largest, these 3 species may be recognized in the following order: *P. thalictri*, *P. infestans*, and *P. phaseoli*. The next group which may be readily identified consists of *P. cinnamomi*, *P. cryptogea*, *P. erythroseptica*, and *P. mexicana*. The writers have not had the opportunity of studying many different strains of these organisms because no such strains were available, yet so far as their experience is concerned, at least 3 of these 4 species should stand for the time being. All 4 of them are scanty sporangia formers under most conditions; *P. cryptogea* has formed no such bodies in all the solutions employed by the senior writer; *P. cinnamomi* is the only one of the 4 capable of producing chlamydospores in solutions; in fact, it has formed only chlamydospores. *P. erythroseptica* and *P. mexicana* differ from the foregoing 2 by the absence of the chlamydospore-forming habit and by their ability to yield sporangia in dilute solutions of nitrates. The chief difference between *P. erythroseptica* and *P. mexicana* lies in the shape of their papillae, but this is a rather dubious distinction, and one that is extremely variable and highly influenced by the environmental conditions. The sporangia of *P. mexicana* may be somewhat larger, but the size factor can not be considered too seriously in making specific distinctions. It would simplify matters if *P. mexicana* were merged with *P. erythroseptica*, and there seems to be no valid reason why this should not be done.

Phytophthora capsici and *P. citrophthora* differ chiefly in the absence of sexual bodies from the latter species. But since there are strains of *P. infestans*, *P. thalictri*, *P. parasitica*, *P. faberi*, etc., described as possessing no sexual bodies, there is no reason why *P. capsici* should not be merged with *P. citrophthora*. The latter organism does not possess sufficiently distinctive specific characters to be very secure anyway, and it is probable that sooner or later it will be found necessary to classify it with the *omnivora* group. The same appears also to apply to *P. nicotianae*, but nothing definite can be said about this until more strains are available for study.

The only way *Phytophthora cactorum* can be distinguished from the *omnivora* group is by means of the position of the antheridia upon the oogonia. Yet, since this does not seem always to occur, and both

paragynous and amphigynous types of antheridia are known to form in *P. cactorum*, this species can be brought still closer to *P. omnivora*. Forms of *P. cactorum* which possess no sexual stage can be placed nowhere else except in *P. omnivora*, and if this species is retained, there can be no doubt that certain of its strains will be classified as *P. cactorum* and others as *P. omnivora*. However, a definite judgment should be suspended on this species, pending the completion of some work now in progress. That *P. fagi* can have no specific standing is reiterated here. The slight differences in the size of sporangia which exist between *P. cactorum* and *P. fagi*, and which have hitherto been considered of sufficient specific value, are no longer tenable because we have seen that while the mean length of *P. cactorum 1* is 32.67 microns and that of *P. fagi* is 36.22 microns, *P. cactorum 2* measures 36.48 microns and *P. cactorum 3* goes up to 39.55 microns. Nor are the differences in the comparative sizes of oogonia large enough to be significant. The pathological relationships are just as unreliable: the numerous physiological races of various fungi and the complex and conflicting relationship of these races to their host plants do not permit an unrestricted use of pathogenicity in taxonomy.

Perhaps the most cosmopolitan and the most common of all Phytophthoras are those that have been gathered by the senior writer into the *omnivora* group. Extremely variable and of wide adaptability, these organisms have been a prolific source of confusion. *P. colocasiae*, *P. faberi*, *P. palmivora*, *P. parasitica*, *P. terrestris*, *P. parasitica rhei*, *P. melongenae*, and perhaps *P. theobromae*, *P. hibernalis*, *P. meadii*, *P. jatrophae*, *P. allii*, and all other so-called new species described from Japan and India and many of them not available in pure-culture form, belong here. The chief reform, therefore, should begin with this group. If any of the foregoing organisms described as distinct species possessed recognizable morphological or physiological characters, there would be no need of a reform. As it is, one may call a certain Phytophthora isolated from coconut or palm as *P. faberi* or *P. palmivora*, but the same culture mixed with a number of other strains of the *omnivora* group can not again be singled out with any degree of certainty. The five colonies of the assumed mutants of *P. parasitica rhei*, as illustrated by the senior writer (*13*), show a wide degree of deviation from the more common type of *P. parasitica* growth. Every macroscopic and microscopic character of this fungus seems to be modified in these five dissociative phases. The typical growth type of *P. parasitica* is a colony of comparatively slow growth, of curly, coagulated patterns, and of bulbous, knotted, variously enlarged and gnarled submerged hyphae. *P. parasitica I and II* illustrate this habit. The *P. faberi*, *P. palmivora* and *P. colocasiae* type of growth consists of a smooth-combed nature with even and regular submerged hyphae. *P. parasitica rhei V* illustrates this mode of growth, while *III* and *IV* show other intermediate stages.

The confusion which now exists in *Phytophthora omnivora* can be largely eliminated if we divide this group into six arbitrary types: 1, *colocasiae-microsporous;* 2, *colocasiae macrosporous;* 3, *faberi-microsporous;* 4, *faberi-macrosporous;* 5, *parasitica-microsporous;* and 6, *parasitica-macrosporous*. The first can be characterized by a smooth-combed colony habit and more elongated but smaller-sized

sporangia; the second is like the first except that the spores are larger; the third has the same colony habit but its sporangia are more spherical; the fourth is like the third, except that the sporangia are larger; the fifth type is characterized by curly, coagulated type of growth and gnarled submerged hyphae, with sporangia either elongated or spherical; the sixth type is like the fifth, except that the sporangia are larger. Such an arrangement provides for most, if not all, of the variations of this unstable group and would save the worker the anomaly of classifying the same organizm under two or more specific names whenever such a fungus happens to dissociate and throw off dissimilar phases. No matter which dissociative stage or which type of strain happened to be isolated by a given worker, it could immediately be classified under a scheme which recognizes that a given culture does not always appear and behave the same way; that it may go through a more or less complicated cyclogeny; that dissociation of a fungus into its different phases can not be indefinitely controlled, and that striking deviations from the common specific path may often occur for various lengths of time. But because these are passive stages they should not be given any permanence by building a true taxonomic pedestal under them.

Blepharospora cambivora should be *Phytophthora cambivora*, and, as such, it may have a comparatively sound taxonomic basis. The lack of papillae on the sporangia of this species is largely a relative matter largely controlled by the environment. When, for instance, this organism was inoculated into green pepper fruits, many of the ensuing sporangia developed well-pronounced papillae, although blunt sporangia were also present in no negligible numbers. But since, under most conditions, the sporangia of *P. cambivora* remain characteristically blunt, this fungus should be considered sufficiently distinctive to be regarded as a new species of Phytophthora.

Phytophthora pini should be retained, at least for the time being. Of all the Pythophthoras grown by the writers this is the only one that forms both sporangia and oogonia in various solutions, a rather unique character, and, like the chlamydospore-forming habit of *P. cinnamomi*, unquestionably distinctive.

Hartley's organisms Nos. 8, 36 and 121 are sufficiently different from all others to merit further study in their native habitat to determine whether they should be described as new species.

SUMMARY

An attempt is made in this paper to compute the comparative value of the size of sporangia obtained under controlled conditions. Measurements of sporangia made by a number of representative workers are tabulated to show the discrepencies which exist in the literature due to the unstandardized methods of investigations, and to the great variability of the individual species. Because even the different strains of the same species vary so much that their sporangia cover the entire scale of sizes known in the reproductive bodies of Phytophthoras, the size factor can not be of primary importance in taxonomy. Oogonia and oospores show similar size variations and are therefore no more valuable in this respect.

Phytophthora infestans, *P. thalictri*, and *P. phaseoli* are taxonomically secure. *P. cinnamomi*, *P. cryptogea*, *P. erythroseptica*, and

P. pini are also distinctive enough to be retained. *P. mexicana* should be merged with *P. erythroseptica* and *P. capsici* with *P. citrophthora*. *Blepharospora cambivora* should be *Phytophthora cambivora*, and *P. fagi* is the same as *P. cactorum*. *P. cactorum* is retained with reservations. *P. arecae*, *P. nicotianae*, and *P. syringae* must be considered of doubtful specific value as classifications until intensive study of a number of strains of these organisms has been made. *P. colocasiae*, *P. faberi*, *P. palmivora*, *P. parasitica*, *P. terrestris*, *P. parasitica rhei*, and *P. melongenae* should be merged with *P. omnivora*; it is probable that *P. theobromae*, *P. allii*, *P. jatrophae*, *P. meadii*, and *P. hibernalis* also belong in that group. Hartley's organisms Nos. 8, 36, and 121 seem to be sufficiently distinctive to merit further study.

LITERATURE CITED

(1) Beach, W. S.
1922. A crown rot of rhubarb caused by Phytophthora cactorum. Penn. Agr. Expt. Sta. Bul. 174, 28 p., illus.

(2) Berlese, A. N.
1898. Icones fungorum omnium hucusque cognitorum ad usum Sylloges Saccardianae. Phycomycetes. 44 p., illus. Patavii.

(3) Carne, W. M.
1927. A brown rot of citrus in Australia (Phytophthora hibernalis n. sp.). Roy. Soc. West. Aust. Jour. and Proc. (1925–26) 12: 13–41.

(4) Clinton, G. P.
1906. Downy mildew, Phytophthora phaseoli Thaxt., of lima beans. Conn. Agr. Expt. Sta. Botanist Rpt. 1905, p. 278–303, illus.

(5) Coleman, L. C.
1910. Diseases of the areca palm (Areca catechu L.). I. Koleroga or rot-disease. Ann. Mycol. 8: [591]–626, illus.

(6) Dastur, J. F.
1916. Phytophthora on Vinca rosea. India Dept. Agr. Mem., Bot. Ser. 8: [233]–242, illus.

(7) Godfrey, G. H.
1923. A Phytophthora footrot of rhubarb. Jour. Agr. Research 23: 1–26, illus.

(8) Gravatt, A. Rathbun.
1925. Direct inoculation of coniferous stems with damping-off fungi. Jour. Agr. Research 30: 327–339, illus.

(9) Hotson, J. W., and Hartge, L.
1923. A disease of tomato caused by Phytophthora mexicana, sp. nov. Phytopathology 13: [520]–531, illus.

(10) Lafferty, H. A., and Pethybridge, G. H.
1922. On a Phytophthora parasitic on apples which has both amphigynous and paragynous antheridia; and on allied species which show the same phenomenon. Roy. Dublin Soc. Sci. Proc. 17: 29–43, illus.

(11) Leonian, L. H.
1922. Stem and fruit blight of peppers caused by Phytophthora capsici, sp. nov. Phytopathology 12: [401]–408, illus.

(12) ———
1925. Physiological studies on the genus Phytophthora. Amer. Jour. Bot. 12: 444–498, illus.

(13) ———
1926. The morphology and the pathogenicity of some Phytophthora mutations. Phytopathology 16: 723–730, illus.

(14) ———
1927. The effect of different hosts upon the sporangia of some Phytophthoras. Phytopathology 17: 483–490, illus.

(15) McMurphy, J.
1916. A Phytopthora on oats. Science (n. s.) 43: 534.

51541—29——6

(16) McRae, W.
1918. PHYTOPHTHORA MEADII, N. SP. ON HEVEA BRASILIENSIS. India Dept. Agr. Mem., Bot. Ser. 9: [219]–273, illus.
(17) Ocfemia, G. O., and Roldan, E. F.
1927. PHYTOPHTHORA BLIGHT OF CITRUS. Amer. Jour. Bot. 14: 1–15, illus.
(18) Pethybridge, G. H.
1913. ON THE ROTTING OF POTATO TUBERS BY A NEW SPECIES OF PHYTOPHTHORA HAVING A METHOD OF SEXUAL REPRODUCTION HITHERTO UNDESCRIBED. Roy. Dublin Soc. Sci. Proc. (n. s.) 13: 529–565, illus. [Reviewed by H. G. MacMillan in Phytopathology 3: 187–189. 1913.]
(19) Petri, L.
1918. STUDI SULLA MALATTIA DEL CASTAGNO DETTA "DELL' INCHIOSTRO." MORFOLOGIA E BIOLOGIA DEL MICELIE PARASSITA. Ann. R. Ist. Super. Forest. Naz. Firenze 3: [151]–185, illus.
(20) Rands, R. D.
1922. STREEPKANKER VAN KANEEL, VEROORZAAKT DOOR PHYTOPHTHORA CINNAMOMI N. SP. (STRIPE CANKER OF CINNAMON, CAUSED BY PHYTHOPHTHORA CINNAMOMI N. SP.). Meded. Inst. Plantenziekten 54, 53 p., illus.
(21) Reinking, O. A.
1923. COMPARATIVE STUDY OF PHYTOPHTHORA FABERI ON COCONUT AND CACAO IN THE PHILIPPINE ISLANDS. Jour. Agr. Research 25: 267–284, illus.
(22) Rose, D. H.
1924. LEATHER ROT OF STRAWBERRIES. Jour. Agr. Research 28: 357–376, illus.
(23) ——— and Lindegren, C. G.
1925. PHYTOPHTHORA ROT OF PEARS AND APPLES. Jour. Agr. Research 30: 463–468, illus.
(24) Rosenbaum, J.
1915. PHYTOPHTHORA DISEASE OF GINSENG. N. Y. Cornell Agr. Expt. Sta. Bul. 363, p. 62–106, illus.
(25) ———
1917. STUDIES OF THE GENUS PHYTOPHTHORA. Jour. Agr. Research 8: 233–276, illus.
(26) Saccardo, P. A.
1888. SYLLOGE FUNGORUM OMNIUM HUCUSQUE COGNITORUM. V. 7, 498-xxx p.
(27) ———
1891. SYLLOGE FUNGORUM OMNIUM HUCUSQUE COGNITORUM. V. 9, 1141 p.
(28) Sherbakoff, C. D.
1917. BUCKEYE ROT OF TOMATO FRUIT. Phytopathology 7: [119]–129.
(29) Smith, R. E., and Smith, E. H.
1906. A NEW FUNGUS OF ECONOMIC IMPORTANCE. Bot. Gaz. 42: 215–221, illus.
(30) Stevens, F. L.
1925. PLANT DISEASE FUNGI. 469 p., illus. New York.
(31) ——— and Plunkett, O. A.
1925. TULIP BLOSSOM BLIGHT. Ill. Agr. Expt. Sta. Bul. 265, p. 299–307, illus.
(32) Sundararaman, S., and Ramakrishnan, T. S.
1924. THE "MAHALI" DISEASE OF COCONUTS IN MALABAR. India Dept. Agr. Mem., Bot. Ser. 13: 87–97, illus.
(33) Tanaka, T.
1917. NEW JAPANESE FUNGI—NOTES AND TRANSLATIONS—II. Mycologia 9: 249–253.
(34) Thurston, H. W., Jr., and Orton, C. R.
A PHYTOPHTHORA PARASITIC ON PEONY. Science (n. s.) 54: 170–171.
(35) Tisdale, W. B., and Kelley, J. G.
1926. A PHYTOPHTHORA DISEASE OF TOBACCO. Fla. Agr. Expt. Sta. Bul. 179, p. 159–219, illus.
(36) Tucker, C. M.
1926. PHYTOPHTHORA BUD ROT OF COCONUT PALMS IN PORTO RICO. Jour. Agr. Research 32: 471–498, illus.

(37) WILSON, G. W.
1907. STUDIES IN NORTH AMERICAN PERONOSPORALES—II. PHYTOPHTHORAE AND RHYSOTHECEAE. Bul. Torrey Bot. Club 34: 387–416.

(38) ———
1914. STUDIES IN NORTH AMERICAN PERONOSPORALES—V. A REVIEW OF THE GENUS PHYTOPHTHORA. Mycologia 6: 54–83, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1929